ALBERT R. MANN LIBRARY
CORNELL UNIVERSITY

CORNELL UNIVERSITY LIBRARY
3 1924 097 732 105

HANN
QK
600
M992
v.94
2006
Oct/Dec.

MYCOTAXON

THE INTERNATIONAL JOURNAL OF FUNGAL TAXONOMY & NOMENCLATURE

Volume 94 *October–December 2005 (2006)*

CONTENTS

Tuber umbilicatum, a new species from China, with a key to the spinose-reticulate spored *Tuber* species **Chen, Juan, Pei-Gui Liu & Yun Wang** 1

Blumenavia toribiotalpaensis: a new species of *Clathraceae* from Jalisco, Mexico **Yalma Luisa Vargas-Rodriguez & J. Antonio Vázquez-García** 7

Studies on Basidiomycetes in Greece 1: The genus *Crepidotus* **Z. Gonou-Zagou & P. Delivorias** 15

The lichen flora of the Termessos National Park in Southwestern Turkey **Özge Tufan, Hüseyın Sümbül & Ayşen Özdemır Türk** 43

Two new species of *Anthracoidea (Ustilaginales)* from China **Lin Guo** 47

A new species of *Lepiota* (*Agaricaceae,* Basidiomycetes) from China **Han-Chen Wang & Zhu-Liang Yang** 51

ITS sequence analysis and ascomatal development of *Pseudogymnoascus roseus* **Y. Jiang & Y.-J. Yao** 55

Morphological and molecular characterization of the mycorrhizas of *Inocybe rufuloides* and *I. splendens* **Mirco Iotti, Mauro Marchetti, Enrico Bonuso & Alessandra Zambonelli** 75

Russula in Himalaya 1: A new species of subgenus *Amoenula* **K. Das, S.L. Miller, J.R. Sharma, P. Sharma, & R.P. Bhatt** 85

Streptopodium passiflorae comb. nov. on *Passiflora rubra* **J.R. Liberato & R.W. Barreto** 89

Type revision of three *Termitomyces* species from India **B.-H. Tang, T.-Z. Wei & Y.-J. Yao** 93

A review of the genus *Gyromitra* (Ascomycota, *Pezizales, Discinaceae*) in Mexico **Rosario Medel** 103

A new species and a new record of *Lepiota* occurring in the Gulf of Mexico area **Leticia Montoya & Victor M. Bandala** 111

The connection of *Dothidotthia aspera* (*Botryosphaeriaceae*) to a hyphomycetous anamorphic fungus, *Thyrostoma negundinis* **Annette W. Ramaley** 127

A new species of *Pertusaria* from Western Australia **Alan W. Archer & John A. Elix** 133

Molecular evidence for the taxonomic status of *Metarhizium taii* and its teleomorph, *Cordyceps taii* (*Hypocreales, Clavicipitaceae*) **Bo Huang, Chunru Li, Richard A. Humber, Kathie T. Hodge, Meizhen Fan & Zengzhi Li** 137

Vamsaprija indica gen. et sp. nov., a bambusicolous, synnematous fungus from India **Puja Gawas & Darbhe Jayarama Bhat** 149

[Content continues inside front cover]

ISSN 0093-4666 MYXNAE 94: 1–412 (2005)
For subscription details, availability on microform, and
availability of articles as photocopies or tear sheets, see back cover

[Content continued from front cover]

Two new species of *Hypogymnia* (*Lecanorales*, Ascomycota) with pruinose lobe tips from China **Xinli Wei & Jiangchun Wei** 155

Discovery and description of a teleomorph for *Leptographium koreanum* **H. Masuya, J.-J. Kim, M. J. Wingfield, Y. Yamaoka, S. Kaneko, C. Breuil & G.-H. Kim** 159

Two parasitic fungi on a new host, *Syringa* (*Oleaceae*) **Ovidiu Constantinescu, Vadim A. Mel'nik & Gerard J.M. Verkley** 175

Further notes on the molecular taxonomy of *Metarhizium* **Bo Huang, Richard A. Humber, Shigui Li, Zengzhi Li & Kathie T. Hodge** 181

The genus *Hymenochaete* (Basidiomycota, Hymenomycetes) in the Hawaiian Islands **Erast Parmasto & Robert L. Gilbertson** 189

A new predatory fungus from China **Dongshen Yang, Weimin Chen, Ying Huang, Minghe Mo & Keqin Zhang** 215

New species of sterile crustose lichens from Australasia **John A. Elix** 219

Some interesting pyrenomycetous fungi on bark of *Quercus* spp. from Spain **Julia Checa & M.N. Blanco** 225

Biogeography and hosts of poroid wood decay fungi in North Carolina: species of *Fomes, Fomitopsis, Fomitella* and *Ganoderma* **L.F. Grand & C.S. Vernia** 231

Two new species of *Ramaria* from southwestern China **Ping Zhang, Zhu-Liang Yang & Zai-Wei Ge** 235

The world's second record of *Neoheteroceras flageoletii* reported from Turkey **Elşad Hüseyin, Faruk Selçuk & Ahmet Şahin** 241

Lewia chlamidosporiformans sp. nov. from *Euphorbia heterophylla* **Bruno S. Vieira & Robert W. Barreto** 245

Weddellomyces turcicus, a new species on a grey *Acarospora* from Turkey **M. Gökhan Halici, Alan Orange & Ahmet Aksoy** 249

Cordyceps spegazzinii sp. nov., a new species of the *C. militaris* group **Monica S. Torres, James F. White, Jr. & Joseph F. Bischoff** 253

A phylogeny of *Ramariopsis* and allied taxa **Ricardo García-Sandoval, Joaqín Cifuentes,Efraín De Luna, Arturo Estrada-Torres & Margarita Villegas** 265

A dichotomous key to *Scutellospora* species (*Gigasporaceae,* Glomeromycota) using morphological characters **Gladstone A. Silva, Leonor C. Maia & Sidney L. Stürmer** 293

New species and phylogenetic relationships of *Hypoxylon* species found in Thailand inferred from the internal transcribed spacer regions of ribosomal DNA sequences **N. Suwannasai, S. Rodtong, S. Thienhirun & A.J.S. Whalley** 303

Tricholoma equestre, the correct name for *T. flavovirens* (*Agaricales*) **H. Deng & Y. -J. Yao** 325

A new species of *Lecanicillium* isolated from the white pine weevil, *Pissodes strobi* **Harry H. Kope & Isabel Leal** 331

Two new species of *Hymenochaetaceae* from eastern China **Yu-Cheng Dai & Bao-Kai Cui** 341

Some entomogenous fungi from Wuyishan and Zhangjiajie nature reserves 2. Three new species of the genus *Hirsutella* **Zongqi Liang, Yanfeng Han, Aiying Liu & Jianzhong Huang** 349

[Content continued on inside back cover]

MYCOTAXON

THE INTERNATIONAL JOURNAL OF FUNGAL TAXONOMY & NOMENCLATURE

A. R. MANN LIBRARY
MAY 2 2 2006
ITHACA, NY 14853

Volume 94, 2005 (2006)

COMPLETE IN ONE VOLUME

CONSISTING OF VI + 412 PAGES INCLUDING FIGURES

EDITOR-IN-CHIEF

Lorelei L. Norvell
editor@mycotaxon.com
Pacific Northwest Mycology Service
6720 NW Skyline Boulevard
Portland, Oregon 97229-1309 USA

ASSOCIATE EDITORS

NOMENCLATURE EDITOR

Shaun R. Pennycook
PennycookS@LandcareResearch.co.nz
Manaaki Whenua Landcare Research
Auckland, New Zealand

BOOK REVIEW EDITOR

David L. Hawksworth
MycoNova, Calle Aguila 12
Colonia La Maliciosa, Mataelpino
ES-28411 Madrid, Spain

FRENCH LANGUAGE EDITOR

Grégoire Hennebert
32 Rue de l'Élevage
B-1340 Ottignies – LLN, Belgium

INDEX EDITOR

Karen D. Gettelman
510 Lake Blvd., Apt. 166
Davis, CA 95616 U.S.A.

EDITORIAL ADVISORY BOARD

Gary J. Samuels, Beltsville, Maryland, USA (1997-2006), Chair
Seppo Huhtinen, Turku, Finland (2000-2005), Past Chair
Donald H. Pfister, Cambridge, Massachusetts, USA (1997-2007)
Wen-Ying Zhuang, Beijing, China (2003-2008)
Carol A. Shearer, Urbana, Illinois, USA (1998-2009)
Sean Ross Pennycook, Auckland, New Zealand (2005-2010)

Published by
MYCOTAXON, LTD, P. O. BOX 264
Ithaca, NY 14581-0264, USA

www.mycotaxon.com
Printed in the United States of America

© Mycotaxon, Ltd, 2005

MYCOTAXON

VOLUME NINETY-FOUR TABLE OF CONTENTS

Tuber umbilicatum, a new species from China, with a key to the spinose-reticulate spored *Tuber* species **Chen, Juan, Pei-Gui Liu & Yun Wang** 1

Blumenavia toribiotalpaensis: a new species of *Clathraceae* from Jalisco, Mexico **Yalma Luisa Vargas-Rodriguez & J. Antonio Vázquez-García** 7

Studies on Basidiomycetes in Greece 1: The genus *Crepidotus* **Z. Gonou-Zagou & P. Delivorias** 15

The lichen flora of the Termessos National Park in Southwestern Turkey **Özge Tufan, Hüseyın Sümbül & Ayşen Özdemır Türk** 43

Two new species of *Anthracoidea (Ustilaginales)* from China **Lin Guo** 47

A new species of *Lepiota* (*Agaricaceae,* Basidiomycetes) from China **Han-Chen Wang & Zhu-Liang Yang** 51

ITS sequence analysis and ascomatal development of *Pseudogymnoascus roseus* **Y. Jiang & Y.-J. Yao** 55

Morphological and molecular characterization of the mycorrhizas of *Inocybe rufuloides* and *I. splendens* **Mirco Iotti, Mauro Marchetti, Enrico Bonuso & Alessandra Zambonelli** 75

Russula in Himalaya 1: A new species of subgenus *Amoenula* **K. Das, S.L. Miller, J.R. Sharma, P. Sharma, & R.P. Bhatt** 85

Streptopodium passiflorae comb. nov. on *Passiflora rubra* **J.R. Liberato & R.W. Barreto** 89

Type revision of three *Termitomyces* species from India **B.-H. Tang, T.-Z. Wei & Y.-J. Yao** 93

A review of the genus *Gyromitra* (Ascomycota, *Pezizales, Discinaceae*) in Mexico **Rosario Medel** 103

A new species and a new record of *Lepiota* occurring in the Gulf of Mexico area **Leticia Montoya & Victor M. Bandala** 111

The connection of *Dothidotthia aspera* (*Botryosphaeriaceae*) to a hyphomycetous anamorphic fungus, *Thyrostoma negundinis* **Annette W. Ramaley** 127

A new species of *Pertusaria* from Western Australia **Alan W. Archer & John A. Elix** 133

Molecular evidence for the taxonomic status of *Metarhizium taii* and its teleomorph, *Cordyceps taii* (*Hypocreales, Clavicipitaceae*) **Bo Huang, Chunru Li, Richard A. Humber, Kathie T. Hodge, Meizhen Fan & Zengzhi Li** 137

Vamsaprija indica gen. et sp. nov., a bambusicolous, synnematous fungus from India **Puja Gawas & Darbhe Jayarama Bhat** 149

Two new species of *Hypogymnia* (*Lecanorales,* Ascomycota) with pruinose lobe tips from China **Xinli Wei & Jiangchun Wei** 155

Discovery and description of a teleomorph for *Leptographium koreanum* **H. Masuya, J.-J. Kim, M. J. Wingfield, Y. Yamaoka, S. Kaneko, C. Breuil & G.-H. Kim** 159

Two parasitic fungi on a new host, *Syringa* (*Oleaceae*)
Ovidiu Constantinescu, Vadim A. Mel'nik & Gerard J.M. Verkley 175

Further notes on the molecular taxonomy of *Metarhizium*
Bo Huang, Richard A. Humber, Shigui Li, Zengzhi Li & Kathie T. Hodge 181

The genus *Hymenochaete* (Basidiomycota, Hymenomycetes) in the Hawaiian Islands **Erast Parmasto & Robert L. Gilbertson** 189

A new predatory fungus from China **Dongshen Yang, Weimin Chen, Ying Huang, Minghe Mo & Keqin Zhang** 215

New species of sterile crustose lichens from Australasia **John A. Elix** 219

Some interesting pyrenomycetous fungi on bark of *Quercus* spp. from Spain
Julia Checa & M.N. Blanco 225

Biogeography and hosts of poroid wood decay fungi in North Carolina: species of *Fomes, Fomitopsis, Fomitella* and *Ganoderma*
L.F. Grand & C.S. Vernia 231

Two new species of *Ramaria* from southwestern China
Ping Zhang, Zhu-Liang Yang & Zai-Wei Ge 235

The world's second record of *Neoheteroceras flageoletii* reported from Turkey
Elşad Hüseyin, Faruk Selçuk & Ahmet Şahin 241

Lewia chlamidosporiformans sp. nov. from *Euphorbia heterophylla*
Bruno S. Vieira & Robert W. Barreto 245

Weddellomyces turcicus, a new species on a grey *Acarospora* from Turkey
M. Gökhan Halici, Alan Orange & Ahmet Aksoy 249

Cordyceps spegazzinii sp. nov., a new species of the *C. militaris* group
Monica S. Torres, James F. White, Jr. & Joseph F. Bischoff 253

A phylogeny of *Ramariopsis* and allied taxa
Ricardo García-Sandoval, Joaqín Cifuentes, Efraín De Luna, Arturo Estrada-Torres & Margarita Villegas 265

A dichotomous key to *Scutellospora* species (*Gigasporaceae,* Glomeromycota) using morphological characters
Gladstone A. Silva, Leonor C. Maia & Sidney L. Stürmer 293

New species and phylogenetic relationships of *Hypoxylon* species found in Thailand inferred from the internal transcribed spacer regions of ribosomal DNA sequences **N. Suwannasai, S. Rodtong, S. Thienhirun & A.J.S. Whalley** 303

Tricholoma equestre, the correct name for *T. flavovirens* (*Agaricales*)
H. Deng & Y. -J. Yao 325

A new species of *Lecanicillium* isolated from the white pine weevil, *Pissodes strobi* **Harry H. Kope & Isabel Leal** 331

Two new species of *Hymenochaetaceae* from eastern China
Yu-Cheng Dai & Bao-Kai Cui 341

Some entomogenous fungi from Wuyishan and Zhangjiajie nature reserves 2. Three new species of the genus *Hirsutella* **Zongqi Liang, Yanfeng Han, Aiying Liu & Jianzhong Huang** 349

Paecilomyces parvosporus, a new species with its relatives from Yunnan Province, China **Yanfeng Han, Zongqi Liang, Huali Chu & Jichuan Kang** 357
Notes on *Otidea* from Xinjiang, China **Wen-Ying Zhuang** 365
Book reviews and notices **David L. Hawksworth** 371

Indices & Information

Nomenclatural novelties proposed in volume 94 *393*
Author index for volume 94 *394*
Reviewers for volume 94 *398*
MYCOTAXON online resources—Index to Fungous and Lichen Taxa, cumulative indices, distributional checklists, past volumes online, information for authors & reviewers, search features *399*
Errata *400*
MYCOTAXON Instructions to authors *401*
Recent books from MYCOTAXON *412*

PUBLICATION DATE FOR VOLUME NINETY-THREE

MYCOTAXON *for* JULY–SEPTEMBER, VOLUME 93 (1–426 + I–VI)

was issued on November 11, 2005

MYCOTAXON

Volume 94, pp. 1–6 October–December 2005

Tuber umbilicatum, a new species from China, with a key to the spinose-reticulate spored *Tuber* species

JUAN CHEN [1,2], PEI-GUI LIU [1*] & YUN WANG[3]

pgliu@mail.kib.ac.cn
1 Kunming Institute of Botany, Chinese Academy of Sciences
Kunming 650204, P. R. China

2 Graduate School of Chinese Academy of Sciences
Beijing 100039, P. R. China

3 Institute of Applied Ecology, Chinese Academy of Sciences
Shenyang 110015, P. R. China

Abstract—*Tuber umbilicatum,* a truffle associated with Pinus yunnanensis in southwestern China, is described and illustrated as a new species. It is characterized by inconspicuously papillate ascomata with an umbilicate basal cavity and ellipsoid ascospores ornamented with spines connected by low ridges to form an alveolate reticulum 6-8 meshes across the spore width. A key to seven *Tuber* species bearing spinose-reticulate spores is presented.

Key words—*Tuberaceae,* truffle, spore ornamentation, hypogeous fungus

Introduction

Since Liu (1985) reported the genus *Tuber* from Shanxi province, China, several additional papers on Chinese truffles have been published. The export of Chinese *Tuber* spp. to Europe has stimulated further interest in members of this genus in China. Currently, up to 20 species, including 10 new species, have been reported in this country (Liu 1985, Tao & Liu 1989, Wang & Li 1991, Hu 1992, Wang et al. 1998, Zhuang 1998, Xu 1999, Wang & He 2002, He et al. 2004, Zhang et al. 2005, Song et al. 2005). During study of truffles associated with *Pinus yunnanensis*, a dominant tree in southwestern China, we found a new truffle, characterized by inconspicuously papillate ascomata with an umbilicate basal cavity and ellipsoid, spinose-reticulate ascospores. To distinguish this new species from others with spinose-reticulate spores, a world-wide key to the seven *Tuber* species with such spores is provided.

Materials and Methods

Macroscopic characters are described from fresh specimen. Microscopic methods of Yang & Zhang (2003) were followed. For scanning electron microscopy (SEM), spores were scraped from the dried gleba onto doubled-sided tape, which mounted directly on an SEM stub, coated with gold-palladium, and examined and photographed with a JEOL JMS-5600LV SEM. Herbaria that provided specimens are abbreviated and cited according to Holmgren et al. (1990), except HKAS (Herbarium of Cryptogams, Kunming Institute of Botany, Chinese Academy of Sciences) and IFS (Herbarium of Institute of Applied Ecology, Chinese Academy of Sciences), two not yet listed in the *Index Herbariorum*.

---

*corresponding author

Taxonomy

Tuber umbilicatum Juan Chen & P. G. Liu, **sp. nov.** **Figs. 1-5**

> *Ascomata ochracea, glabra vel subglabrabasi umbilicata. Peridium bistratum, 320-500 μm crassum: stratum exterius pseudoparenchymaticum, stratum interius hyphi intertextis. Gleba solida, purpureobruneola vel griseobruneola, venis albis. Asci sporis 1-4 (6). Ascosporae ellipsoideae, ochraceae, 21-40 × 14-32 μm, spinulis 3-5 μm altis, reticulato alveolato < 1μm alto connexis ornatae. Holotypus hic designatus: HKAS 44316.*
>
> ***Etymology:*** Latin, *umbilicatum,* in reference to the umbilicate depression of the ascomata.

Ascomata (Fig. 1) 1.2-1.9 cm broad, globose, with an umbilicate depression at the base, surface smooth or with minute papillae up to 50 μm high, pale yellow, becoming yellow-brown or brown when dried. **Peridium** (Fig. 2) brittle, peeling easily from the gleba, mostly 320-500 μm thick, composed of two layers: outer layer 90-250 μm thick, pseudoparenchymatous, composed of subglobose to subangular, yellow-brown cells 7-16 × 5-11 μm, the walls 1-3 μm thick; inner layer 150-400 μm thick, of intricately interwoven, hyaline hyphae 2-5 μm in diam, the walls thin to somewhat thickened. **Gleba** purple-brown or grey-brown with pink tint at maturity, marbled with numerous, narrow, branching, white veins radiating from the basal cavity.

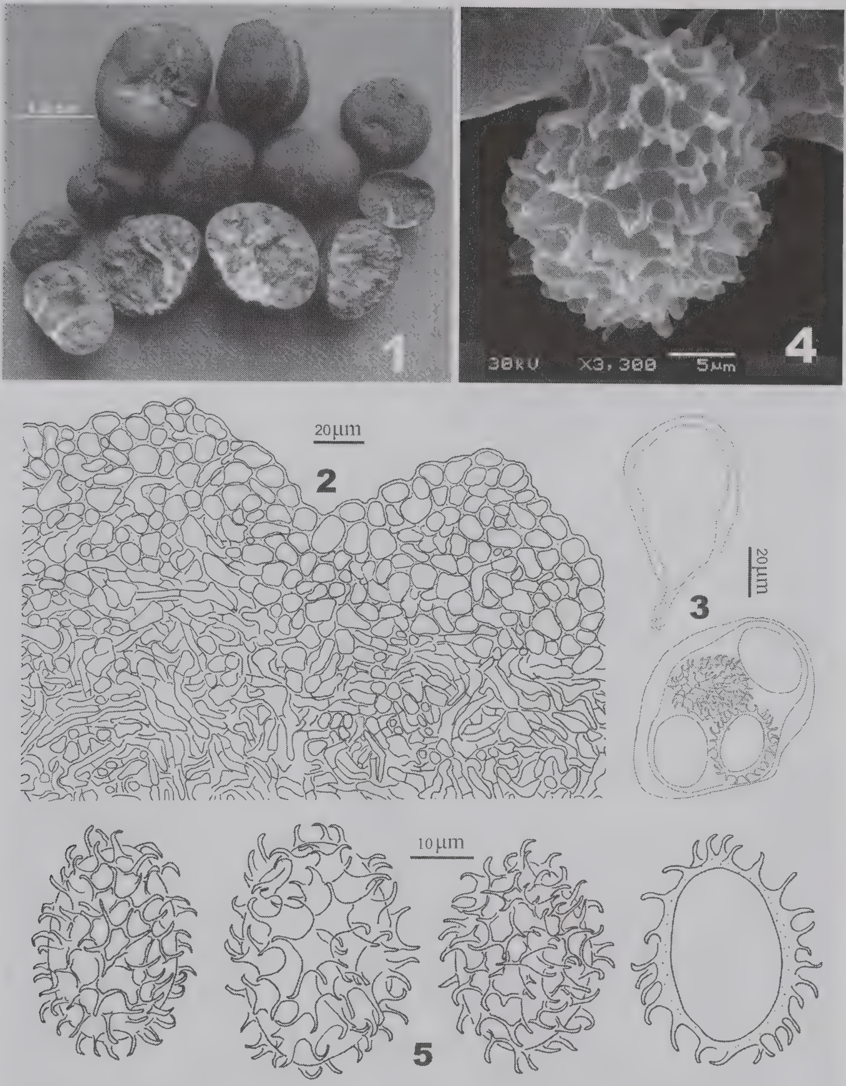
Asci (Fig. 3) 50-83 × 37-67 μm excluding the stalk, globose to subglobose, ellipsoid or irregular, sessile or sometimes with a short stalk, 1-4 (-6) spored. **Ascospores** (Fig. 4, 5) ellipsoid, yellow-brown at maturity, the walls up to 2 μm thick, in 1-spored asci (28-) 33-40 × 20-32 μm excluding ornamentation, 2-spored asci (23-) 25-37 × 17-26 μm, 3-spored asci (21-) 23-33 (-36) × 17-23 μm, 4-spored asci 21-30 (-32) × 15-22 (-25) μm, 5-spored asci 21-26 × 14-20 μm; Q = (1-) 1.2-1.6 (-1.8), **Q** = 1.4 ± 0.13; ornamentation of spines 3-5 (-6) μm tall connected by an alveolate reticulum < 1 μm tall, the alveolae 3-6 (-7) × 2.5-5 μm, 6-8 across the spore width and (6-) 7-10 along the spore length.

Habitat: Hypogeous under *Pinus yunnanensis.*

> **Specimen examined—CHINA: Yunnan province**, Chengjiang county, Tigu Village, elev. 1900-2000 m, 31 Oct. 2003, Juan Chen 145 (HKAS 44316 – holotype).

Discussion—The surface of the ascomata of *T. umbilicatum* is glabrous to subglabrous to the naked eye, but very fine papillae can be seen with a stereomicroscope. These papillae give the peridium a wavy outer edge under the compound microscope. The combination of umbilicate, nearly smooth ascomata and spinose-reticulate spores is distinctive.

Species of *Tuber* can be divided on the basis of spore ornamentation into three groups: 1) spiny (representative species: *T. melanosporum* Vittad.), 2) reticulate (representative species: *T. borchii* Vittad.) and 3) spinose-reticulate (representative species:



Figs. 1-5 *Tuber umbilicatum* (HKAS 44316, Holotype)
1. Fresh ascomata; 2. Vertical section of the peridium; 3. Asci;
4. SEM of spore, showing the detail of ornamentation; 5. Ascospores.

T. spinoreticulatum Uecker & Burds.). *T. umbilicatum* belongs to the *T. spinoreticulatum* group, which includes *T. taiyuanense* B. Liu, *T. pseudoexcavatum* Y. Wang et al. and *T. huidongense* Y. Wang, known only from China; *T. lyonii* Butters (=*T. texense* Heimsch) and *T. spinoreticulatum* from North America; and *T. malacodermum* E. Fisch. from

Germany and Switzerland (Fischer 1923, Heimsch 1958, Uecker & Burdsall 1977, Liu 1985, Wang et al. 1998, Wang & He 2002, Trappe et al. 1996). These species can be difficult to distinguish because of their morphological similarity. Accordingly, to clarify differences among these species we re-examined the holotypes of *T. huidongense* (IFS 89923) and *T. texense* (OSC 42353), isotype of *T. spinoreticulatum* (OSC 38860), a neotype of *T. taiyuanense* (HMAS 75888) and two specimens of *T. pseudoexcavatum* (HKAS 47617, 41313). Ascomata and SEM photomicrographs of spores are illustrated in Figs. 6-11. Although we did not obtain specimens of *T. malacodermum*, Fischer's (1923) description as confirmed by study of the type collection by J. M. Trappe (personal communication) showed it to have a pseudoparenchymatous peridium composed of cells inflated up to 20-70 μm broad. A key to the spinose-reticulate species is presented here, based on these studies.

Key to species with spinose-reticulate spores

1. Peridium with rounded cells inflated to 20-70 μm . . . . ***T. malacodermum***
1. Peridium of interwoven hyphae or with rounded cells mostly < 20 μm broad . . 2

2. Ascomata with a basal cavity 3
2. Ascomata without a basal cavity 5

3. Peridium smooth or with inconspicuous papillae 10-50 μm high; spores ellipsoid, Q > 1.3 . . . . ***T. umbilicatum***
3. Peridium with conspicuous warts 100-150 μm high; spores mostly broadly ellipsoid, Q ≤ 1.3 4

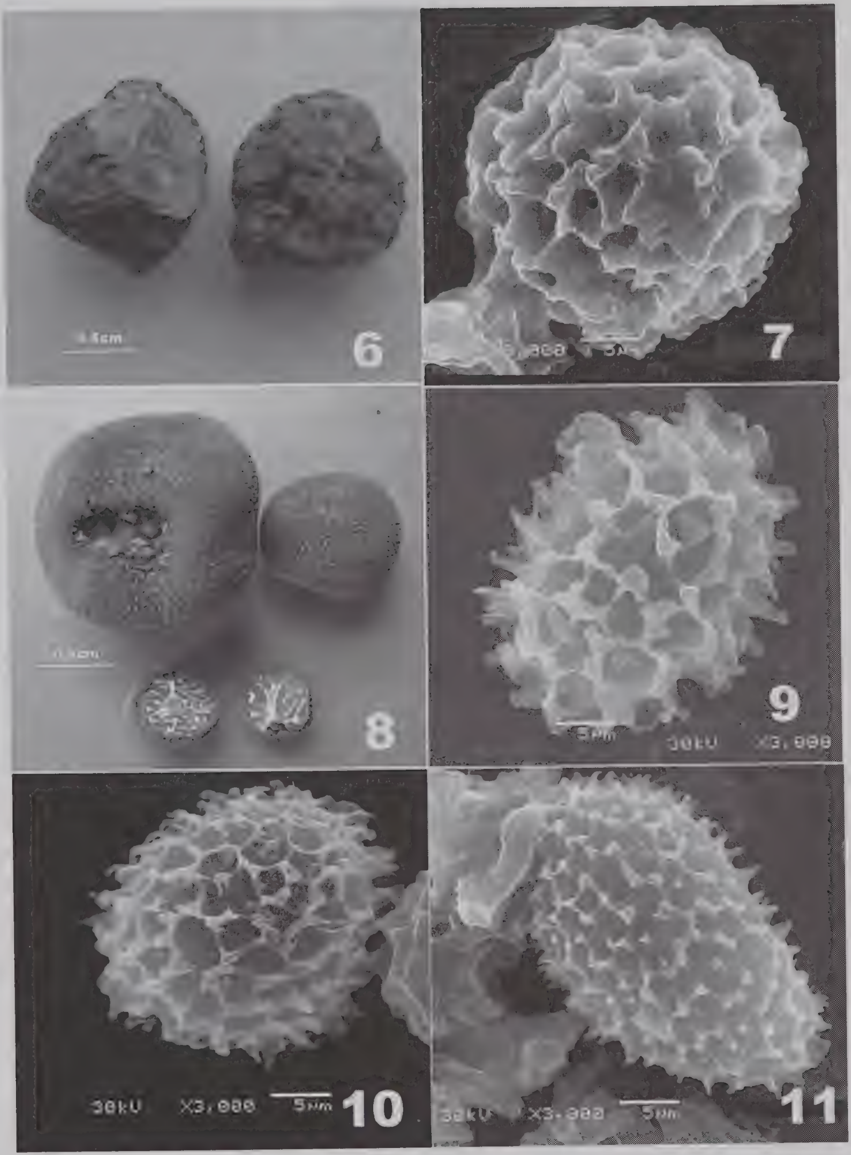
4. Cavity conspicuous; asci 1-8 spored, sessile . . . . ***T. pseudoexcavatum***
4. Cavity inconspicuous; asci 1-4 spored, stipitate . . . . ***T. spinoreticulatum***

5. Ascomata smooth; ascospores broadly ellipsoid, Q < 1.3, mostly 1-1.25. . . . . ***T. taiyuanense***
5. Ascomata with minute papillae up to 55 μm high; ascospores ellipsoid, Q > 1.3, mostly 1.3-1.6. 6

6. Spores with longer spines mostly > 3 μm long, reticulum complete and regular, meshes size bigger 4-8 × 4-6 μm . . . . ***T. huidongense***
6. Spores with shorter spines 2-3 μm long, reticulum partial to complete, meshes size smaller 3-6 × 2-4 μm. . . . . ***T. lyonii*** (=*T. texense*)

Acknowledgments

Prof. James Trappe and Dr. Michael Castellano reviewed the manuscript and offered invaluable comments and suggestions. Specimens were generously lent to us by the herbaria of the Department of Botany & Plant Pathology, Oregon State University, Royal Botanic Gardens, Kew, and Institute of Microbiology, Chinese Academy of Sciences. Sincere gratitude is offered to Prof. M. Zang, Dr. Zhu L. Yang and Mrs. X. H. Wang, Kunming Institute of Botany, for their valuable suggestions on earlier version of the manuscript. Many thanks to my colleagues, F. Q. Yu, H. D. Zheng and J. Y. Chen for providing morphological photos. The study was supported in part by National Science Foundation of China (No. 30470011), the Knowledge Innovation Program of Chinese Academy of Sciences (No. KSCXZ-1-09-06 & KSCXZ-SW-101C) and the Nature Science Foundation of Yunnan province (No. 2004C0050M).


6
7
8
9
5μm
30kU
X3,000
10
30kU
X3,000
5μm
11
30kU
X3,000
5μm

Figs. 6-11. *T. taiyuanense* (HMAS 75888, neotype): 6. Ascomata; 7. SEM micrograph of ascospore. *T. pseudoexcavatum* (HKAS 47617): 8. Ascomata. *T. huidongense* (IFS 89923, holotype): 9. Ascospore. *T. spinoreticulatum* (OSC 38860, isotype): 10. Ascospore. *T. texense* (OSC 42353, holotype): 11. Ascospore.

Literature cited

Fischer E. 1923. Zur Systematik der Schweizerischen Trüffeln aus den Gruppen von *Tuber excavatum* und *rufum*. Verhandlungen Naturforschenden Gesellschaft Basel 35: 34-50.

He XY, Li HM, Wang Y. 2004. *Tuber zhongdianense* sp. nov. from China. Mycotaxon 90: 213-216.

Heimsch C. 1958. The first recorded truffle from Texas. Mycologia 50: 657-660.

Holmgren PK, Holmgren NH, Barnett LC. 1990. Index Herbariorum: part I: herbaria of the world. 8th edition, New York Botanical Garden: New York. 693 pp.

Hu HT. 1992. *Tuber formosanum* sp. nov. and its mycorrhizal associations. Quarterly Journal of the Experimental Forest, National Taiwan University 6: 79-86.

Liu B.1985. New species and new records of hypogeous fungi from China (I). Acta Mycologica Sinica 4: 84-89.

Song MS, Cao JZ, Yao YJ. 2005. Occurrence of *Tuber aestivum* in China. Mycotaxon 91: 75-80.

Tao K, Liu B. 1989. A new species of the genus *Tuber* from China. Journal of Shanxi University (Natural Science Edition) 12: 215-218.

Trappe JM, Jumpponen AM, Cázares E. 1996. NATS truffle and truffle-like fungi 5: *Tuber lyonii* (=*T. texense*), with a key to the spiny-spored *Tuber* species groups. Mycotaxon 60: 365-372.

Uecker FA, Burdsall HH. 1977. *Tuber spinoreticulatum*, a new truffle from Maryland. Mycologia 69: 626-630.

Wang Y, He XY. 2002. *Tuber huidongense* sp. nov. from China. Mycotaxon 83: 191-194.

Wang Y, Li ZP. 1991. A new species of the genus *Tuber* from China. Acta Mycologica Sinica 10: 263-265. (in Chinese).

Wang Y, Moreno G, Riousset LJ, Manjón JL, Riousset G, Fourré G, Massimo GD, García Montero LG, Díez J. 1998. *Tuber pseudoexcavatum* sp. nov., a new species from China commercialized in Spain, France and Italy with additional comments on Chinese truffles. Cryptogamie Mycologie 19: 113-120.

Xu AS. 1999. Notes on the genus of *Tuber* from Tibet. Mycosystema 18: 361-365. (in Chinese).

Yang ZL, Zhang LF. 2003. Type studies on *Clitocybe macrospora* and *Xerula furfuracea* var. *bispora*. Mycotaxon 88: 447-454.

Zhang LF, Yang ZL. Song DS. 2005. A phylogenetic study of commercial Chinese truffle and their allies: Taxonomic implications. FEMS Microbiology Letters 245: 85-92.

Zhuang WY. 1998. A list of discomycetes in China. Mycotaxon 67: 365-390.

MYCOTAXON

Volume 94, pp. 7–14 October–December 2005

Blumenavia toribiotalpaensis: a new species of *Clathraceae* from Jalisco, Mexico

[1]Yalma L. Vargas-Rodriguez & [2]J. Antonio Vázquez-García

yvarga1@lsu.edu

[1]107 Life Sciences Building, Dept. of Biological Sciences, Louisiana State University
Baton Rouge 70803, Louisiana, U.S.

[2]Depto. de Botánica y Zoología, CUCBA, Universidad de Guadalajara
km 15 carr. Guadalajara-Nogales, Las Agujas
Nextipac, 45110, Zapopan, Jalisco, Mexico

Abstract—A new stink horn species, *Blumenavia toribiotalpaensis* sp. nov. *(Clathraceae)* from Mexico, is described and illustrated. *Blumenavia toribiotalpaensis* differs from previously described species of the genus by its larger receptacle and basidiospores, distribution of the gleba over the upper half of the columns and presence of no more than four columns.

Key words—Basidiomycota, phalloid, systematics, Talpa de Allende

Introduction

During the course of studies of the distribution and demography of a recently discovered *Acer saccharum* subsp. *skutchii* (cloud forest sugar maple) population in Jalisco, Mexico (Vargas-Rodriguez 2005), we collected fungal specimens with novel features. A survey of the literature revealed that the specimens are an undescribed species of *Blumenavia* (*Clathraceae*).

The genus *Blumenavia* was established to accommodate *B. rhacodes* Möller (Möller 1895). Currently, two species, *B. rhacodes* and *B. angolensis* (Welw. & Curr.) Dring, are included in the genus (Dring 1980). The genus is characterized primarily by the small number of columns that lack transverse arms. In addition, the columns are free at the base and united at their apices with glebifers consisting of membranes attached by one side to each of the two inner angles of the column (Dring 1980, Sáenz 1980).

The new *Blumenavia* species occurs in a pine-cloud forest transition considered a Tertiary refuge (Vargas-Rodriguez 2005). The subtropical montane cloud forest is unique in having high plant species richness and includes a number of endangered and relict plants, comparable only to certain Asian forests (Graham 1999, Vargas-Rodriguez 2005). The forest contains temperate disjunct tree genera with East Asia and East North America, such as *Acer, Magnolia, Carpinus, Cornus, Fraxinus, Juglans, Tilia,* and *Ostrya* (Graham 1999, Vázquez-García et al. 2000). This exceptional community is proposed for protection as a biosphere reserve with 3,000 inhabitants supporting the movement

(Vargas-Rodriguez 2005). The discovery of the novel *Blumenavia* species increases our knowledge and relevance of the biota of this unique region.

Materials and Methods

We collected fruiting bodies at different stages of development in a pine-montane cloud forest transition (1,800 m a.s.l.), near Talpa de Allende, Jalisco, Mexico, among fallen leaves under the canopy of adult *Pinus* spp. and *Carpinus caroliniana* trees. A second collection was made a year later in the same area and a third one in 2005. Five fruiting bodies were fixed in FAA solution (five parts 40% formaldehyde: five parts glacial acetic acid: 90 parts 95% ethyl alcohol) and seven were dried. A free hand cross section was made to one egg. Spores were mounted on slides in lactophenol and in 3% KOH and examined with a NIKON Microphot compound microscope using differential interference contrast and bright field optical systems. Hand-cut sections of the columns were made from a sample that had been fixed in FAA for nine months. Sections were dehydrated with ethanol, critical point dried, mounted, and coated with gold:palladium 60:40 in an Edwards S-150 sputter coater. We used a Cambridge S-260 scanning electron microscopy (SEM) for observation.

Taxonomic Description

Blumenavia toribiotalpaensis **Vargas-Rodriguez sp. nov.** **FIGS. 1-14**

> *Ovum fulvum, superficies interdum findens aquamis fulvis angularibus, elipsoidale 2.2-3.8 cm longum x 2.1-3.9 cm altum; adhaesae albae rhizomorphae 1-1.5 cm diametro. Receptaculum expansum 12.1-15.3 cm altum, 3.8-5.6 cm longum, colore vario ab albulo ad dilutum bombax, cylindrale (or cylindratum) ex 3 vel 4 columnis robustis constans; columnis 0.9-1.1 cm diametro ex parte gracillima, 1.9-2.1 cm ex parte latissima, conjunctis superne, inferne liberis, cum sulco in superfacie; semicirculares sectione, constantes ex 9 tubis compositis in 3 ordinibus ab canali abaxiali; proximi canali sunt 5 tubuli circulares sectione in material nova; medius ordo constat ex 3 latioribus tubis, polygonalibus sectione; atque ex singulari magna polygonali tuba constat ordo extremus ab canali abaxiali. Columnae tela glebifera incrassata in facie interna informante cristam per anterior-laterales angulos columnae, glebifera incrassata marginibus unita, crista blebam ferente. Gleba coercita intra laceratam glebiferam cristam, sita in superior parte columnarum, atro-brunnescenti-olivacea, aroma simili piscibus mortuis et nauseosa. Basidiosporae 3.8-4.2 x 1.7-1.9 µm. Associati basidiocarpis siti Scarabaei (Staphilinidae et Leiodidae) intra brachia et muscae (Tephritidae) in gleba maturorum basidiocarporum.*

Egg pale brown, outer surface sometimes cracking into angular brown scales, ellipsoidal, 2.2-3.8 cm wide, 2.1-3.9 cm high, gelatinous layer 5-7 mm thick, traversed by peridial sutures corresponding to each of the four columns, immature glebal mass about 4 mm diameter among columns, glebiferous tissue separated by a cavity opened in one extreme and joined to the column only by the opposite extreme, medullar zone rectangular-like in shape; attached white rhizomorphs 1-1.5 mm in diameter. Expanded receptacle 12.1-15.3 cm high and 3.8-5.6 cm wide, whitish to pale beige, cylindrical, with 3 to 4 robust columns; columns 0.9-1.1 cm diameter at the thinnest part to 1.9-2.1 cm at the widest part, united above, free below with a groove in the outer surface, semicircular in section,

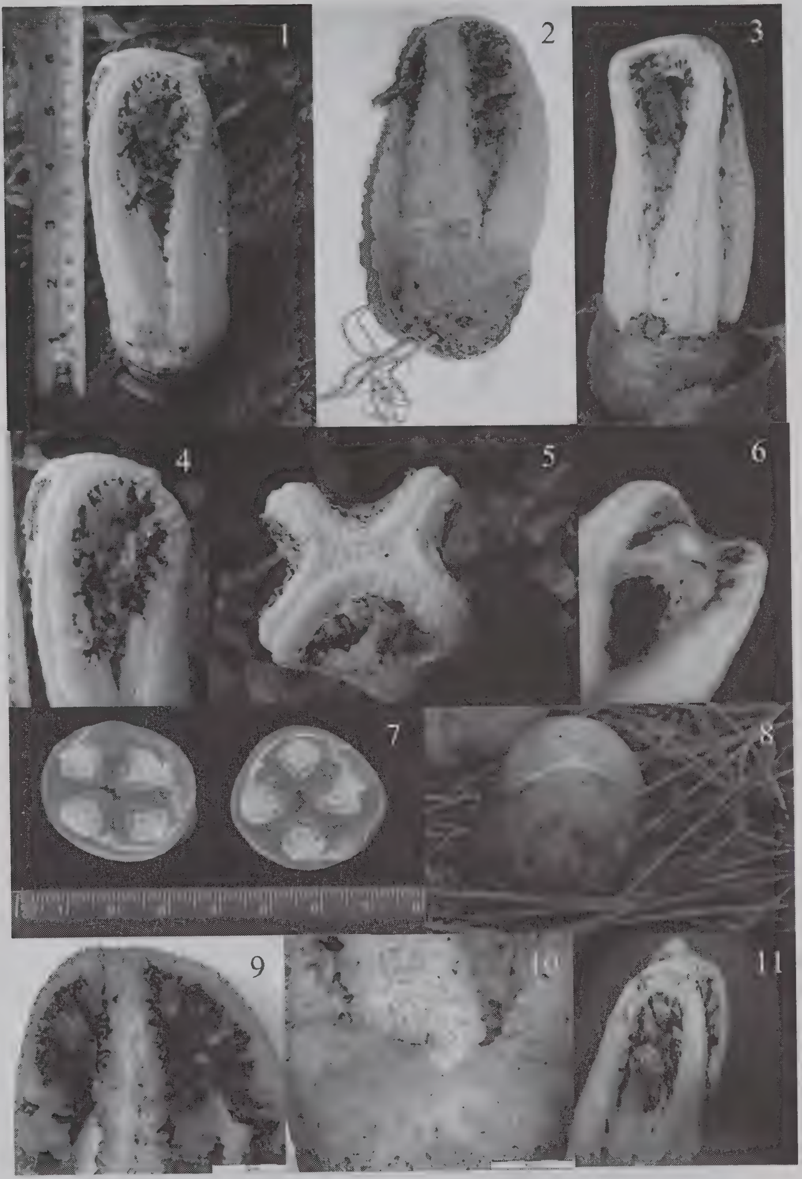


Figure 1-11. *Blumenavia toribiotalpaensis*, IBUG 422a. **1,** Habitat photograph showing receptacle and volva. **2,** Receptacle, volva, and rhizomorphs from specimen preserved in FAA. IBUG 456, **3,** Receptacle and volva. IBUG 422a. **4, 5, 9, 10,** Detail of gleba, showing its distribution over the upper half of the receptacle. IBUG 456, **6 & 11,** Detail of gleba. Yalma L. Vargas-Rodriguez 455, 7 **& 8.** Freehand cross section of an egg. IBUG 422a, **9,** Detail of the volva from specimen preserved in FAA.

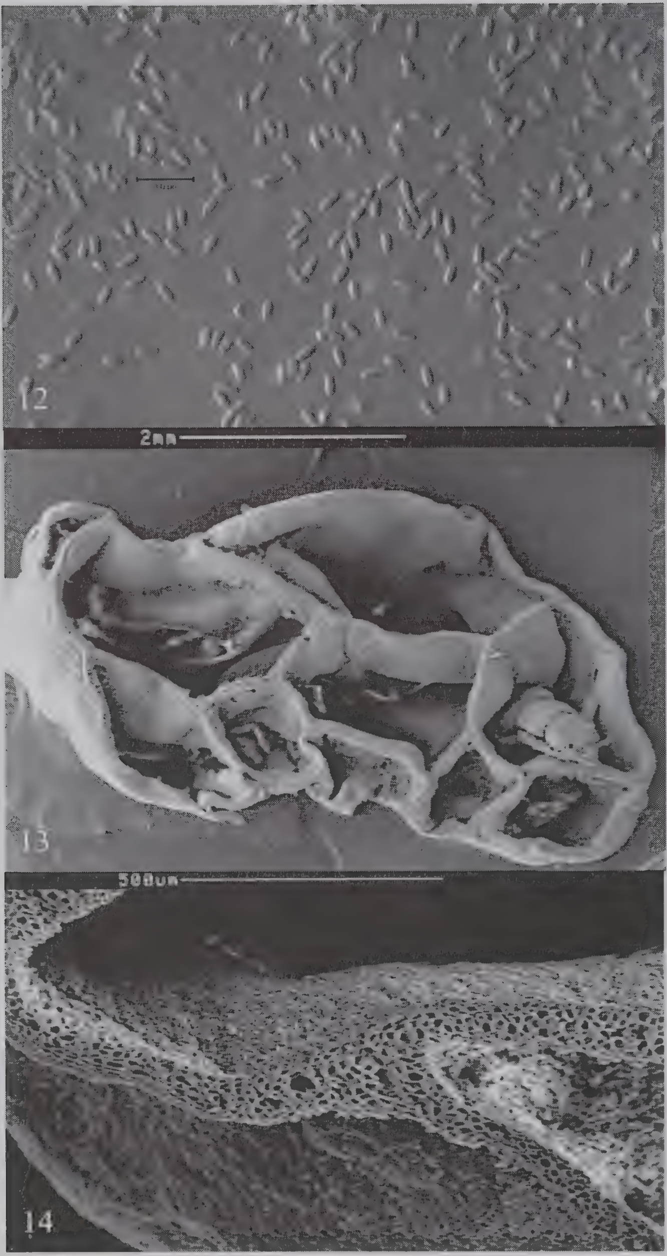


Figure 12-14. *Blumenavia toribiotalpaensis*, LSUM 422b. **12,** Basidiospores. **13,** Sections of the arm showing number and arrangement of tubes and associated staphylinid beetle. **14,** Inner (upper side) and outer (bottom) arm surfaces.

comprised of 9 tubes arranged in three ranks from the abaxial groove; nearest to the groove are five small tubes, circular in section in fresh material; the middle rank consists of three wider tubes, polygonal in section; and a single, large polygonal tube comprises the outermost rank from the abaxial groove (Fig. 13). Columns with a thickened glebiferous tissue on the inner surface forming a crest along the anterior-lateral angles of the column, glebiferous tissue united to the arms by the edges, crest bearing the gleba. Gleba restricted to the lacerate glebiferous crest, situated on the upper half of the columns, dark olive-brownish, odor of dead fish, nauseous. Basidiospores 3.8-4.2 x 1.7-1.9 µm, cylindrical, hyaline in KOH. Beetles [Staphilinidae (Fig. 13), Leiodidae] and flies (Tephritidae) associated with basidiocarps, beetles located inside the arms reach the interior through holes in basidiocarps, holes appear in decaying basidiocarps; flies located in gleba of mature basidiocarps.

> **Specimen examined** — HOLOTYPE **here designated**. **MEXICO, JALISCO: Talpa de Allende municipality**, pine-cloud forest (*Acer-Podocarpus-Abies*) transition, "Ojo de Agua del Cuervo" ("Crow spring") locality, west of Cumbre de Los Arrastrados (20°11"N; 105°16"W), 1800 m a.s.l., on *Pinus* spp. fallen trunk and debris, 10 Sep 2002, *Yalma L. Vargas-Rodriguez 240*, with *Javier Curiel, J. Antonio Vázquez-García* and *Toribio Quintero Moro*, dry specimen (BPI); 10 Sep 2002, *Yalma L. Vargas-Rodriguez 240a*, with *Javier Curiel, J. Antonio Vázquez-García* and *Toribio Quintero Moro*, dry specimen (IBUG) (Holmgren et al. 1990).
>
> PARATYPE **here designated**. **MEXICO, JALISCO: Talpa de Allende municipality**, pine-cloud forest (*Acer-Podocarpus-Abies*) transition, "Ojo de Agua del Cuervo" ("Crow spring") locality, west of Cumbre de Los Arrastrados (20°11"N; 105°16"W), 1800 m a.s.l., on fallen leaves of *Pinus* spp. and *Carpinus caroliniana*, 14 Sep 2003, *Yalma L. Vargas-Rodriguez 422b, 423a, 423b, 424a* and *J. Antonio Vázquez-García*, FAA preserved material (LSUM); 14 Sep 2003, *Yalma L. Vargas-Rodriguez 422a* and *J. Antonio Vázquez-García*, FAA preserved material (IBUG); 13 Sep 2005, *Yalma L. Vargas-Rodriguez 454, 456, 462* and *J. Antonio Vázquez-García*, dry specimen (IBUG); 13 Sep 2005, *Yalma L. Vargas-Rodriguez 459, 463* and *J. Antonio Vázquez-García*, dry specimen (LSUM).

Etymology—From the Latin *talpaensis*, referring to the municipality where the fungus was collected and *toribio*, referring to Toribio Quintero Moro, a remarkable forest conservationist. He has promoted the protection of the Talpa de Allende forests by collecting 3,000 signatures from Talpa de Allende habitants and petitioning state and federal Mexican authorities for the creation of a new biosphere reserve in the area.

Known distribution—Jalisco: Only known from the type locality. The species is not common in the area; individuals are patchy distributed along 100 m. The species was not previously known by local people. Only three other species (*Clathrus crispus, C. cancellatus*, and *C. ruber*) of the *Clathraceae* family are known for the Jalisco state and these do not co-occur with *Blumenavia toribiotalpaensis*. This is the first record of the genus for western Mexico and the second one for the country.

Habit and habitat—Occasionally gregarious. Among plant leaves and debris, under *Pinus* spp., *Carpinus caroliniana* and *Acer saccharum* subsp. *skutchii* canopy, in transitional pine forest to montane cloud forest.

Discussion

Blumenavia differs from similar stink horn genera in the glebifer form. Especially notable is the unique gleba borne on lateral flaps of tissue that is lacking in *Clathrus*, *Laternea*, and *Ligiella*.

The differences between *Blumenavia toribiotalpaensis* and the two previously described species include the distribution of the gleba and the size of the receptacle and basidiospores (Table 1). The gleba is distributed along half the length of the column in *B. toribiotalpaensis;* in *B. rhacodes* the gleba is present over the entire length of the column and in *B. angolensis* restricted to the upper quarter or one third of the receptacle. The receptacle and basidiospores are larger in *B. toribiotalpaensis* than in the other two species (Table 1).

Table 1. Differences among species of *Blumenavia*

	Blumenavia rhacodes	*Blumenavia angolensis*	*Blumenavia toribiotalpaensis*
Expanded receptacle	8.5-13x8 cm	10x3 cm	12.1-15.3x3.8-5.6 cm
Receptacle color	Clear orange to yellow	White	Whitish to pale beige
Sections of columns; number of tubes	Triangular and trapezoidal; about 10 tubes	Subtriangular or quadrangular; about 6 tubes	Semicircular; 9 tubes
Number of columns	3-6	3-5	3-4
Groove along outer side of column	Present	Lacking	Present
Basidiospores	3-4x1-1.5 μm	3-3.5x1.5 μm	3.8-4.2 x 1.7-1.9 μm
Gleba distribution	Entire length of the column	Upper quarter or one third of the receptacle	Upper half
Habitat and distribution	In coffee plantations in cloud forest in Mexico. Mexico: Veracruz; The Caribbean: Trinidad; South America: Brazil.	Habitat not noted. Africa: Angola, Tanzania, South Africa; US: Texas; The Caribbean: Puerto Rico; South America: Brazil.	Pine-montane cloud forest transition. Mexico: Jalisco.

Mature specimens of *B. toribiotalpaensis* usually have four columns but one immature specimen has only three. The white columns in fresh specimens separate *B. toribiotalpaensis* from *B. rhacodes* with orange-yellow columns; *B. angolensis* however, also has white columns. The spongy texture of the columns of *B. toribiotalpaensis* distinguishes this species from the more rigid, less spongy texture of *B. angolensis.* Number, shape and arrangement of tubes in transverse sections of columns differ, being more numerous in *B. toribiotalpaensis* than in *B. angolensis,* with a semicircular shape, and arranged differently from *B. rhacodes* (Table 1).

Habitat and known geographical distribution differ among the three *Blumenavia* species. Although both *B. toribiotalpaensis* and *B. rhacodes* are known from Mexico, *B. rhacodes* has been found in coffee plantations established under the canopy of cloud forest trees at 1300 m a.s.l. in Teocelo, Veracruz, Mexico (López et al. 1981), while *B. toribiotalpaensis* occurs at higher elevations (1800 m a.s.l.) in the transition of pine and cloud forest. In Mexico, *B. rhacodes* is also known from Xalapa, Veracruz (Calonge et al. 2004). This is the first record of the genus for western Mexico and is the second for the country, which was previously found in eastern Mexico (Veracruz state). Individuals of *Blumenavia* species have low density in Mexico. *Blumenavia angolensis* is known from Angola, Tanzania, South Africa and Brazil, although the habitats were not reported (Dring 1980) (Table 1).

Acknowledgments

The authors are grateful to Meredith Blackwell for her helpful comments to the manuscript and for her technical support. Margaret C. Henk kindly helped with the photomicrographs. Diane Ferguson and Vesna Karaman helped with the photographs. Richard Warga wrote the Latin description. We acknowledge the assistance of David Farr (USDA National Fungus Collection, BPI), who made material of *B. rhacodes* available for comparison. Erick Soto-Cantu, Heather Passmore and Angeles Vargas acquired literature needed in the study. We especially appreciate the helpful suggestions of Laura Guzmán-Dávalos, Silvia Cappello Garcia and Joaquín Cifuentes throughout the study and the peer reviews of Peter Roberts and Kentaro Hosaka.

Literature Cited

Calonge FD, Guzmán G, Ramírez-Guillén F. 2004. Observaciones sobre los Gasteromycetes de México depositados en los herbarios XAL y XALU. Boletín de la Sociedad Micológica de Madrid 28: 337-371.

Dring DM. 1980. Contributions towards a rational arrangement of the *Clathraceae*. Kew Bulletin 35: 1-96.

Graham A. 1999. Late Cretaceous and Cenozoic history of North American vegetation. Oxford University Press, New York, New York, USA.

Holmgren PK, Holmgren NH, Barnett LC. 1990. Index herbariorum. Part I. Ed. 8. Regnum Vegetation 120: 1-693.

López A, Martínez D, García J. 1981. Adiciones al conocimiento de los Phallales del estado de Veracruz. Boletín de la Sociedad Mexicana de Micología 16: 109-116.

Möller A. 1895. Botanische Mittheilungen aus den Tropen. Heft 7. Jena: G. Fisher, 152 pp.

Sáenz JA. 1980. *Ligiella*, a new genus for the *Clathraceae*. Mycologia 72: 338-349.

Vargas-Rodriguez YL. 2005. Ecology of disjunct cloud forest sugar maple populations (*Acer saccharum* subsp. *skutchii*) in North and Central America. Master of Science Thesis, Louisiana State University, Baton Rouge, Louisiana, USA.

Vázquez-García JA, Vargas-Rodriguez YL, Aragon F. 2000. Descubrimiento de un bosque de *Acer-Podocarpus-Abies* en el municipio de Talpa de Allende, Jalisco, México. Boletín del Instituto de Botánica, Universidad de Guadalajara 7: 159-183.

MYCOTAXON

Volume 94, pp. 15–42 October–December 2005

Studies on Basidiomycetes in Greece 1: The genus *Crepidotus*

Z. Gonou-Zagou & P. Delivorias

zgonou@biol.uoa.gr panadeli@biol.uoa.gr
University of Athens, Faculty of Biology, Department of Ecology & Systematics
Panepistimioupoli, GR–157 84 Athens, Greece

Abstract—The diversity of *Crepidotus* in the Eastern Mediterranean region is poorly known, and data from Greece are scarce. The present work aims at the record and study of the diversity of the genus in Greece and at the contribution to the knowledge of the distribution of the genus in Europe. Forty-four collections have been examined and ten taxa have been identified. *Crepidotus autochthonus, C. lundellii, C luteolus, C. subverrucisporus* and *C. applanatus* var. *subglobiger* are newly recorded from Greece and most taxa are recorded on new substrates for both Greece and Europe. Detailed descriptions, ecological notes and taxonomical comments on all studied taxa are given.

Key words—lignicolous fungi, mycodiversity, biodiversity, taxonomy, *Crepidotaceae*

Introduction

Crepidotus is a distinct and well-defined genus, and although most species remain not yet completely documented and clarified, several monographic works, as well as critical revisions and regional studies based mainly on morphological studies, provide comprehensive systematic treatment of many species (Singer 1947; Pilát 1948; Hesler & Smith 1965; Singer 1973; Watling & Gregory 1989; Nordstein 1990; Stangl et al. 1991; Senn-Irlet 1995; Senn-Irlet & De Meijer 1998; Aime 2001; Krisai-Greilhuber et al. 2002; Bandala & Montoya 2002a, 2002b, 2004). The systematic position of *Crepidotus* was until very recently debatable, as it was placed either in family *Crepidotaceae* (Moser 1978; Jülich 1981; Singer 1986; Hawksworth et al. 1995), *Strophariaceae* (Kühner 1980) or *Cortinariaceae* (Bas 1988; Kirk et al. 2001). Recent phylogenetic analyses (Aime 1999; Moncalvo et al. 2002; Aime et al. 2005) allow to redefine the *Crepidotaceae* within a broader phylogenetic framework of the agarics, and the genera *Crepidotus* and *Simocybe* are better supported in family *Crepidotaceae* s.s., which represents a separate lineage of dark-spored euagarics.

In the Mediterranean region some studies on *Crepidotus* exist, mainly concerning N. Africa (Malençon & Bertault 1975), Spain (Ortega & Buendia 1989) and Italy (Lonati 2000). However, the knowledge of the diversity of *Crepidotus* in the Eastern Mediterranean region is poor, as few papers have been published and most of these are not easily accessible. The data from Greece are scarce. To date, only seven taxa were recorded from Greece, usually without descriptions, and most collected very few times (Diapoulis 1939; Maire & Politis 1940; Avtzis & Diamandis 1988; Minter 1988; Diamandis & Perlerou 1990; Pantidou 1991; Diamandis 1992; Zervakis et al. 1998; Dimou et al. 2002a; Dimou et al. 2002b; Konstantinidis 2002; Polemis et al. 2002). Few papers from neighboring countries have been recently published, such as Croatia (Tkalčec & Mešić 2003) and Turkey (Öztürk et al. 2003; Ersel & Solak 2004; Sesli & Denchev 2005). Most of the above mentioned papers are floristic studies, lacking detailed descriptions or exsiccata.

The aim of the present study is to provide a better understanding of the biodiversity of the genus in Greece, concerning its morphology (including the range and importance of its variability), ecology and chorology. Collections have been made from various regions of central and southern continental Greece, mainly from coniferous and deciduous forests, as well as riparian and maquis vegetation.

The following ten taxa are identified and described in this work: *C. applanatus* var. *subglobiger, C. autochthonus, C. calolepis, C. cesatii* var. *cesatii, C. epibryus, C. lundellii, C. luteolus, C. mollis, C. subverrucisporus* and *C. variabilis.*

Materials and methods

Microscopical observations were made in bright field or phase contrast using a standard light transmission microscope. Sections of dried material were mounted in 3% KOH, with or without the addition of Phloxine. All measurements were made under 1000× magnification. At least 20 spores and 10 basidia and cheilocystidia were measured per specimen. The spores were measured from the surface of the pilei or from a spore deposit (when available). The spore sizes are given in approximation to 0.5 µm, with extreme values given in parentheses, followed by the length-width ratio of the spores (Q). Habitat references in the descriptions refer exclusively to the collected material. Greek localities are transcribed into latin according to ISO 843: 1997 (E). Authorities' abbreviations are in accordance to *Authors of Fungal Names* by Kirk & Ansell (1992). We have adopted the infrageneric classification proposed by Senn-Irlet (1995).

Material from other collectors or researchers (published or unpublished), was examined when available. The specimens collected from the authors are deposited in the Mycological Herbarium of the University of Athens (ATHU–M).

Taxonomic descriptions

Crepidotus (Fr.: Fr.) Staude 1857

Crepidotus subgenus *Crepidotus*

Crepidotus calolepis (Fr.) P. Karst. **Figs 1a–b; 9b, d; 11g**

Crepidotus calolepis (Fr.) P. Karst., Bidr. Känn. Finl. Nat. Folk 32: 414 (1879); *Crepidotus mollis* var. *calolepis* (Fr.) Pilát, Ann. Hist. –Nat. Mus. Natl. Hung., n.s. 2B: 74 (1940); *Crepidotus mollis* ssp. *calolepis* (Fr.) Nordstein, Synopsis Fungorum (Oslo) 2: 67 (1990).

Pileus 10–70 mm, semicircular to flabelliform, convex to plano-convex, laterally or almost laterally attached to the substrate, with incurved and later even margin, surface viscid-sticky to dry, densely minutely tomentose-scaly with yellowish brown to brown fibrillose scales on a dirty whitish, cream to ochre-yellowish background, consistency tough, elastic. **Lamellae** whitish in young specimens, then spotted brownish and finally uniformly ochre-brown to cinnamon, moderately crowded, emarginately adnate, margin minutely fimbriate, remaining whitish. **Stipe** absent or rudimentary. **Spore print** yellowish brown.

Basidiospores 7.5–11.0 × 5.5–7.0 µm, Q = 1.21–1.69, ellipsoid, amygdaliform in side view, smooth, yellowish brown in KOH, thick-walled, apex obtuse, depressed or occasionally mucronate; in some spores inner wall curving inwards at apex, resembling a callus or an indistinct germ pore when accompanied by a small apical depression (Figs 1a, 9b). **Basidia** 25–32 × 7–9 µm, cylindrical-clavate, 4-spored. **Cheilocystidia** 25–65 × 4–14 µm, clavate, cylindrical, irregularly cylindrical, somewhat fusiform to narrowly lageniform, sometimes branched, apex obtuse, often subcapitate (Fig. 1b), frequently embedded in gelatinous material entirely covering the lamellar edge. **Pleurocystidia** absent but pleurocystidioid-like bodies present in some specimens, clavate, with a short, apical or rarely lateral, finger-like protuberance (Fig. 11g). **Lamellar trama** often gelatinized. **Pileipellis** with an underlying layer of parallel hyphae, 3–5 µm wide, hyaline, not encrusted, and an upper layer of parallel to somewhat ascending hyphae, 4–12 µm wide, hyaline to pale ochraceous, with encrusting zebra-like pigment, amongst which ascending scale-forming hyphae, 4–15 µm wide, brown to dark brown, thin- to somewhat thick-walled, short-celled, with strongly encrusting, zebra-like pigment (Fig. 9d), in some specimens with markedly large, plate-like encrustations. **Pileal trama** partly gelatinized, gelatinous layer underlying the pileipellis usually distinct, up to 200 µm thick, but occasionally thin, rudimentary, and hence difficultly observed. **Secretory hyphae** occasionally present in pileipellis, pileal trama and lamellar trama, hyaline to golden yellow in KOH. **Clamp connections** absent in all tissues.

Habitat: Solitary to gregarious on standing or fallen trunks and branches of coniferous or deciduous trees.

Specimens examined — Mt. Taygetos, Messinia, on wood of *Platanus orientalis,* 29 Nov. 1968, Pantidou, ATHU–M 1071 (as *C. mollis*); Mt. Kandilio, Pagontas-Prokopi, Evvoia, forest of *Pinus* sp., on fallen branches of *Pinus* sp., 13 Dec. 1986, Gonou, ATHU–M 3781; Mt. Taygetos, Messinia, forest of *Pinus nigra* & *Abies cephalonica,* on fallen branches, 17 Nov. 1997, Gonou, ATHU–M 3782; Mt. Aroania, Zarouchla, Achaïa, forest of *A. cephalonica,* on stump of *A. cephalonica,* 27 Nov. 1997, Delivorias, ATHU–M 3970; Mt. Parnitha, Attiki, forest of *A. cephalonica,* on fallen branches of *A. cephalonica,* 4 Dec. 1997, Gonou, ATHU–M 3783; Mt. Vardousia, Artotina, Fokida, forest of *P. nigra* & *Abies* sp., on fallen branches, 26 Sep. 1999, Gonou, ATHU–M 5107; Mt. Parnonas, Agios Petros, Arkadia, forest of *Castanea sativa* & *Quercus* sp., on fallen branches, 21 Nov. 1999, Gonou, ATHU–M 5105; river Agrafiotis, Epiniana, Evrytania, riparian vegetation, on living trunk and branches of *Pl. orientalis,* 15 Oct. 2000, Delivorias, ATHU–M 5122; Gardiki, Fthiotida, forest of *Alnus glutinosa,* 15 Oct. 2000, Dimou, 731; Ano Chora, Nafpaktia, Aitoloakarnania, dead trunk of *Pl. orientalis,* 22 Nov. 2001, Dimou, 988; Mt. Tymfristos, Agios Nikolaos, Evrytania, forest of *Pl. orientalis, Quercus* sp. & *C. sativa,* on trunk base of *Pl. orientalis,* 8 Nov. 2003, Gonou, ATHU–M 5108; Aetos, Messinia, on branches of *Pl. orientalis,* 6 Dec. 2003, Kapsanaki, ATHU–M 5111; Mt. Liakoura, Granitsa, Evrytania, forest of *Abies borisii-regis,* on fallen twigs of *A. borisii-regis,* 27 Sep. 2004, Delivorias, ATHU–M 5161; Mt. Liakoura, Limeri, Evrytania, forest of *P. nigra,* on fallen branches of *P. nigra,* 13 Oct. 2004, Delivorias, ATHU–M 5174; Mt. Tymfristos, Agios Nikolaos, Evrytania, mixed forest of *Pl. orientalis, Quercus frainetto, C. sativa* and *A. borisii-regis,* on branches of *Pl. orientalis,* 23 Oct. 2004, Gonou, ATHU–M 5169; Mt. Liakoura, Granitsa, Evrytania, forest road, on living branches of *Pl. orientalis,* 11 Nov. 2004, Delivorias, ATHU–M 5175; Mt. Liakoura, Granitsa, Evrytania, forest of *A. borisii-regis* and *Pl. orientalis,* on fallen trunk of *Pl. orientalis,* 11 Nov. 2004, Delivorias, ATHU–M 5178.

Remarks: *C. calolepis* is very closely related to *C. mollis* and is considered by some authors as a variety or subspecies of the latter (Pilát 1948; Nordstein 1990). Others (Singer 1973; Watling & Gregory 1989; Senn-Irlet 1995, Bandala & Montoya 2004) consider the two taxa distinct at a specific level. Both species are characterized by the presence of a gelatinous layer in the pileal trama, considered to be more developed in *C. mollis* and less developed or absent in *C. calolepis*. This, however, has not been considered a reliable distinguishing feature (Nordstein 1990, Senn-Irlet 1995). We have accepted the species-concept of *C. calolepis* as portrayed by Senn-Irlet (1995), who has performed the most detailed work on the European species of the genus. According to this concept, *C. calolepis* is distinguished from *C. mollis* by the somewhat broader basidiospores and the presence of yellowish brown, fibrillose scales on the pileal surface formed by brownish hyphae with encrusting pigment. The pileal surface of *C. mollis* is glabrous or with scattered innate fibrils that may form indistinct pale scales, but the hyphae of the pileipellis are not pigmented and never heavily encrusted.

Our collections include specimens with strongly fibrillose-scaly pilei and specimens with almost or completely glabrous pilei, as well as many transitional forms. We cross-examined the morphology of the pileal surface, the structure of the pileipellis and the spore dimensions and concluded that two distinct forms exist amongst our collections. The first form is characterized by whitish pilei, glabrous throughout or with few fibrillose scales at the centre, in which the pileipellis consists of hyaline to pale yellowish hyphae with minute encrustations and the spores are consistently narrower (only exceptionally exceeding 6 μm in width and never more than 6.5 μm). The second form is characterized by yellowish to yellowish-brown, minutely to strongly fibrillose-scaly pilei, often throughout, in which the scale-forming hyphae of the pileipellis are consistently more or less strongly pigmented (yellowish brown to dark reddish brown in KOH) and in all cases heavily encrusted, and the spores are broader (most exceeding 6 μm and frequently reaching 7 μm in width). We identified the former as *C. mollis* and the latter as *C. calolepis.*

The Mediterranean variety *C. calolepis* var. *squamulosus* (Cout.) Senn-Irlet is not clear to us. It is reported by Senn-Irlet (1995) to have slightly larger basidiospores than var. *calolepis* (8.5–12 × 6–7.5 μm versus 7.5–10 × 5–7 μm) and broader scale-forming hyphae (up to 22 μm wide, instead of 14 μm wide). The spore-size in our specimens holds an intermediate position between the two varieties, as, in most collections, a significant portion of the spores exceed 10 μm in length, but we have not measured any spores larger than 11 μm in length or 7 μm in width. Also, we have not encountered any scale-forming hyphae broader than 15 μm. Although the spores in our specimens are slightly larger than those reported by Senn-Irlet, this has been reported by other authors as well (Bandala & Montoya 2004) and cannot be considered a significant enough deviance to justify a distinction at a variety level. We have therefore concluded that all of our specimens must belong to a single taxon, i.e. var. *calolepis.* Lonati (1993) reports *C. mollis* var. *squamulosus* Cout. with a spore size of 7–10 × 5–6 (–6.5) µm and scale-forming hyphae 3–8 µm wide. Judging on his description, Lonati's *C. mollis* var. *squamulosus* must in fact be *C. calolepis* var. *calolepis.* Malençon & Bertault (1975) also report having found *C. mollis* var. *squamulosus,* but they distinguish it from *C. calolepis* on the grounds that the latter represents a small-sized species with a non-gelatinized or little-gelatinized pileipellis, features that are not considered diagnostic (Nordstein 1990). They make no reference to the spore-size or the structure of the pileipellis. It is unclear to us whether their *C. mollis* var. *squamulosus* represents *C. calolepis* var. *calolepis* or var. *squamulosus.*

An interesting deviant feature in our examined collections is that many basidiospores present a curving of the inner wall at the spore apex, with the outer wall either remaining obtuse and thus giving the impression of a callus-like formation, or having a small depression, resembling an indistinct germ pore (Figs 1a, c; 9a–b). This was constantly observed in all examined specimens of both *C. mollis* and *C. calolepis* and should be considered characteristic for the two species. Singer (1973) also reports similar characteristics. Senn-Irlet (1995) refers that in SEM analyses, spores of *C. calolepis* and *C. mollis* reveal a small apical depression, which may be interpreted as an apical thinning of the wall, yet neither a truncate spore apex or an apical thinning was visible under the light microscope. When describing in general the spores of the genus, Singer (1986) reports that the spores may occasionally have an indistinct callus or rarely an indistinct germ pore.

In some specimens of *C. calolepis,* as well as *C. mollis,* the lamellar trama and margin are distinctly gelatinous, the gelatinous material often covering the lamellar edge entirely. The presence of this material is possibly related to environmental humidity.

Differentiatied pleurocystidioid-like bodies were observed in the hymenium of some *C. calolepis* specimens, being more frequent near the margin. Singer (1986) reports that cystidioles may often be present on the sides of the lamellae in *Crepidotus* species and Hesler & Smith (1965) go as far as to acknowledge these elements as pleurocystidia. Senn-Irlet (1995) observed such bodies in *C. cesatii* and interpreted them as abnormalities induced by drought and therefore of no taxonomic significance. Apart from *C. calolepis,* we have also encountered such bodies in specimens of *C. subverrucisporus.* It is doubtful that these elements are of taxonomical significance in either case, as their presence is not constant and could not be correlated with any other deviant feature.

C. calolepis seems to be by far the most common representative of the genus in Greece, as we have collected it on several substrates from various locations. However, it was formerly recorded only by Maire & Politis (1940) on a stump of *Pinus halepensis* and dead trunks of *Platanus* sp., collections dating back to 1904 and 1906. On the other hand, the closely related *C. mollis* is recorded a number of times in the literature (Diapoulis 1939; Maire & Politis 1940; Pantidou 1991; Zervakis et al. 1998; Dimou et al. 2002a). We examined a collection of Pantidou (ATHU–M 1071), identified as *C. mollis.* It consists of a single, well-preserved specimen. The pileus is covered almost throughout with minute fibrillose scales and the scale-forming hyphae are yellowish brown to brown with strongly encrusting pigment. The spore dimensions fit accurately to our measurements of other specimens of *C. calolepis.* We have therefore concluded that this specimen formerly attributed to *C. mollis* in fact belongs to *C. calolepis* as here

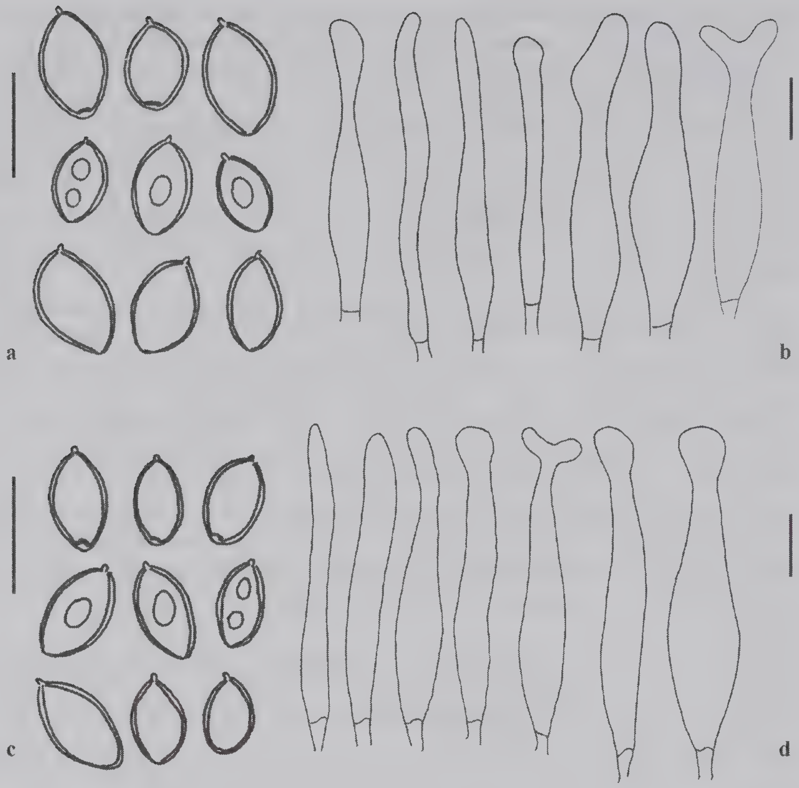


Fig. 1. a–b. *C. calolepis:* **a.** basidiospores, **b.** cheilocystidia, **c–d.** *C. mollis:* **c.** basidiospores, **d.** cheilocystidia. Scale bars = 10 µm.

interpreted. We also examined three specimens collected by Dimou (pers. com.), two of which he had identified as *C. mollis* var. *calolepis* (731, 988, unpubl.), and one as *C. mollis* var. *mollis* (961, unpubl.), and we concur with his judgement. Typical forms of *C. mollis* and *C. calolepis* are easily distinguished from one another in the field, but we have encountered many non-typical specimens, transitional in appearance, that could easily be misidentified if not carefully examined under the microscope. The presence of fibrillose scales on the pileal surface can be variable in abundance, and specimens collected in wet weather often have apparently glabrous pilei, to the naked eye, as stressed by Bandala & Montoya (2004). It is our conviction that *C. calolepis* may have occasionally been mistaken for *C. mollis* in the past and is in fact much more common in Greece than the latter. Pilát (1948) states that *C. calolepis* is more common in dry areas or drier inland climates. This may explain the frequent occurrence of *C. calolepis* in Greece, a country with a drier climate in comparison to most European countries.

Most authors (Malençon & Bertault 1975, Ortega & Buendia 1989, Watling & Gregory 1989, Nordstein 1990, Senn-Irlet 1995, Breitenbach & Kränzlin 2000, Krisai-Greilhuber et al. 2002) report either or both *C. mollis* and *C. calolepis* solely on wood of broad-leaved trees. Scarce reports exist from wood of coniferous trees (Maire & Politis

1940, Bandala & Montoya 2004). In Greece, *C. calolepis* has been reported on a stump of *Pinus halepensis* (Maire & Politis 1940), now it is newly recorded on branches of *Abies cephalonica, Abies borisii-regis* and *Pinus nigra*. Of the material collected in this work, seven collections of *C. calolepis,* as well as two collections of *C. mollis,* were found on wood of coniferous trees (*Abies* and *Pinus*), which have not been recorded as substrates of either species in Europe. It has also been recently collected on branches of *Alnus glutinosa* (Dimou, pers. com.). Furthermore, eight collections of *C. calolepis* were found on wood of *Platanus orientalis,* a host also not included in the substrates of this species for Europe (Senn-Irlet 1995).

Crepidotus mollis (Schaeff.: Fr.) Staude Figs 1c–d; 9a, c; 11b, d, k

Crepidotus mollis (Schaeff.: Fr.) Staude, Schwämme Mitteldeutschl. 25: 71 (1857)

Pileus 10–50 mm, semicircular to flabelliform, convex to plano-convex, laterally or almost laterally attached to the substrate, with incurved and later even margin, surface viscid-sticky to dry, white to cream, glabrous to minutely fibrillose, forming scattered, indistinct fibrillose scales, more evident in dried specimens, consistency tough, elastic. **Lamellae** whitish in young specimens, then spotted brownish and finally uniformly ochre-brown to cinnamon, moderately crowded, emarginately adnate, margin minutely fimbriate, remaining whitish. **Stipe** absent or rudimentary.

Basidiospores 7.0–10.0 × 5.0–6.0 (–6.5) µm, Q = 1.42–1.82, ellipsoid, amygdaliform in side view, smooth, yellowish brown in KOH, thick-walled, usually with one and less often two large oil drops as well as few small ones, apex often mucronate and thin-walled, in some spores inner wall folding inwards resembling a callus or an indistinct germ pore (Figs 1c, 9a). **Basidia** 12–30 × 6–9 µm, cylindrical-clavate, 4-spored. **Cheilocystidia** 32–55 × 6–10 µm, irregularly cylindrical, lageniform to slightly fusiform, apex obtuse, sometimes subcapitate, rarely branched or septate, frequently embedded in gelatinous material entirely covering the lamellar edge (Fig. 1d, 11b). Basidioles, basidia and cheilocystidia rarely with golden-yellow, smooth content (Fig. 11d). **Lamellar trama** seldom gelatinized. **Pileipellis** with an underlying layer of parallel hyphae, 3–5 µm wide, hyaline, not encrusted, and an upper layer of hyaline to pale yellowish hyphae, 4–12 µm wide, with granular or minutely encrusting pigment but never heavily encrusted or strongly pigmented (Fig. 9c). **Pileal trama** partly gelatinized, gelatinous layer underlying the pileipellis, usually well-developed. **Secretory hyphae** occasionally present in pileipellis, pileal trama and lamellar trama (Fig. 11k), scarce to abundant, hyaline to golden yellow in KOH. **Clamp connections** absent in all tissues.

Habitat: Gregarious on living or dead trunks and branches of *Abies borisii-regis* and *Platanus orientalis.*

Specimens examined — Mt. Zygourolivado, Pefkofyto, Karditsa, forest of *Abies borisii-regis,* on a fallen trunk of *A. borisii-regis,* 19 Sep. 1999, Delivorias, ATHU–M 5117; Mt. Zygourolivado, Pefkofyto, Karditsa, forest of *A. borisii-regis,* on a fallen trunk of *A. borisii-regis,* 17 Nov. 2001, Delivorias, ATHU–M 5123; Tatoi, Attiki, on fallen twigs of a deciduous tree, Dimou, 961; Mt. Tymfristos, Agios Nikolaos, Evrytania, mixed forest of *Platanus orientalis, Quercus frainetto, Castanea sativa* and *A. borisii-regis,* on branches of *P. orientalis,* 23 Oct. 2004, Gonou, ATHU–M 5170; Mt. Liakoura, Granitsa, Evrytania, forest of *A. borisii-regis* and *P. orientalis,* on living trunk and branches of *P. orientalis,* 11 Nov. 2004, Delivorias, ATHU–M 5177; Mt. Liakoura, Granitsa, Evrytania, forest of *A.*

borisii-regis and *P. orientalis*, on dead branches of *P. orientalis*, 11 Nov. 2004, Delivorias, ATHU–M 5180.

Remarks: The aforementioned collections are those we have encountered to fit *C. mollis*. For further notes on *C. mollis* and comparison with *C. calolepis* see remarks under *C. calolepis*.

Secretory hyphae were observed in abundance in the lamellar trama of basidiocarps from collection ATHU–M 5123. However, in basidiocarps of ATHU–M 5117, collected two years earlier from the same trunk, these hyphae were scarce, and in specimen 961 collected by Dimou, no secretory hyphae were found. The presence of these hyphae is most likely due to environmental conditions or the stage of maturity of the basidiocarps, and is of little or no taxonomical merit. Similar secretory hyphae were also observed in some specimens of *C. calolepis*.

C. mollis is newly recorded for Greece on *Abies borisii-regis*. According to the substrate's list of the species in Europe (Senn-Irlet 1995), there is only one reference of the species from *Platanus* and none from conifers.

Crepidotus subgenus *Dochmiopus* (Pat.) Pilát, 1948

Section *Dochmiopus*

Crepidotus applanatus var. ***subglobiger*** Singer **Figs 2a–d; 10h; 11h-j**

Crepidotus applanatus var. *subglobiger* Singer, Nova Hedwigia Beih. 44: 478 (1973)

Pileus 5–30 mm, semicircular, flabelliform to petaloid, convex to plano-convex, laterally attached to the substrate, margin incurved to even, hygrophanous, translucently striate at margin when wet, surface smooth, somewhat felty at point of attachment, pure white to pale cream, becoming yellowish brown in dried specimens. **Lamellae** whitish in young specimens, then buff to snuff brown, moderately distant, adnate to decurrent, margin minutely fimbriate, remaining whitish (observed under lens). **Stipe** rudimentary, with white tomentum on the substrate.

Basidiospores (5.0–) 5.5–6.5 (–7.0) × (5.0–) 5.5–6.0 (7.0) µm, Q = 1.00–1.20, globose to subglobose, yellowish brown in KOH, with pinkish content, finely verrucose, with a perispore (Figs 2a, 10h). **Basidia** 18–26 × 6–8 µm, cylindrical-clavate, 4-spored, with basal clamp, content with numerous small oil drops. **Cheilocystidia** 45–110 × 5–12 µm, variable, typically cylindrical to narrowly lageniform, rather often subcapitate or curved at apex, some branched, some septate, in some cases with two septa on the same cystidium, hyaline, thin-walled but rather often thick-walled at the medial part, in some cystidia markedly so, up to 3 µm thick (Fig. 2b, d; 11h-j). **Pleurocystidia** absent but abundant pleurocystidioid-like bodies present, 14–22 × 5–6 µm, irregularly cylindrical to clavate, often curved, twisted or constricted, frequently with an apical to lateral, finger-like protuberance (Fig. 2c). **Pileipellis** a cutis, hyphae 4–8 µm wide, hyaline or with pale yellowish, diffuse to somewhat granular intracellular pigment, occasional hyphal ends exerting as pileocystidia, 30–70 × 6–9 µm, narrowly lageniform, cylindrical to subcapitate, hyaline. Secretory hyphae present in pileipellis, pileal trama and lamellar trama. **Clamp connections** present in all tissues.

Habitat: Scattered on a rotten fallen trunk of *Picea abies*.

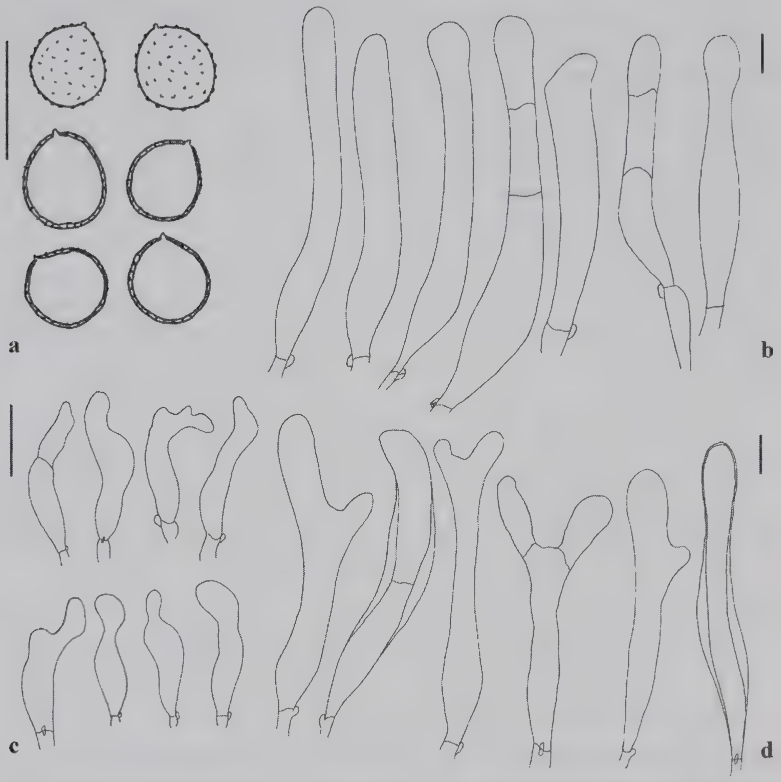


Fig. 2. *C. applanatus* var. *subglobiger:* **a.** basidiopores, **b, d.** cheilocystidia, **c.** pleurocystidoid bodies. Scale bars = 10 µm.

Specimens examined — W. Rodopi Mts., Elatia, Drama, alt. 1550 m, on a fallen trunk of *Picea abies,* 5 Oct. 2005, Gonou & Floudas, ATHU–M 5332.

Remarks: *C. applanatus* var. *subglobiger* is distinguished from the typical variety by the shape of the cheilocystidia which are longer, narrowly lageniform to cylindrical instead of clavate to capitate. Furthermore, var. *applanatus* prefers hardwoods, whereas var. *subglobiger* seems to be restricted to coniferous wood.

We have collected this taxon only once, from a *Picea* forest in Northern Greece. Microscopic examination revealed a few deviant features, such as the presence of septa on many cystidia, and even, in many occasions, two septa on the same cystidium. This was not a constant feature however, as showed by examination of different lamellar margins from the same basidiocarp. In one lamella the cheilocystidia were almost predominately septate, whereas in a nearby lamella the septate cystidia were scarce to almost absent. Also, many cystidia with a markedly thick-walled medial part were encountered, with the remaining cystidium being thin- to slightly thick-walled. Finally, we observed many branched cystidia at the apices, either forked or laterally branched, in most cases with two, rarely three, branches. It was suprisingly difficult to find basidia, although most specimens were fully mature, with abundant basidiospores in all preparations. We encountered instead many basidioles and pleurocystidioid bodies, these most probably being abnormally developed basidia. All the above mentioned abnormalities, if they be such, might be induced by environmental conditions.

Hesler & Smith (1965) described two varieties of *C. applanatus* based on the morphology of the cheilocystidia: var. *phragmocystidiosus* with septate cystidia and var. *diversus* with branched or knobbed cystidia. These varieties are considered conspecific by Aime (2001) with *C. applanatus* s. Joss. She concludes, after detailed examination, that cheilocystidia in this taxon are, under the influence of environmental conditions, capable of secondary growth that can alter their shape and size as well as the number of septa per cystidium. This, however, does not seem to be the case in our specimens, as the septate and non-septate cystidia are morphologically similar. We agree, nevertheless, with Aime's deduction that taxonomic delineation in *Crepidotus* cannot be based on cystidial morphology alone, as the form of the cheilocystidia may vary greatly within a single taxon and, as observed in this case, even within individual collections.

C. applanatus has been reported twice from Greece, from a fallen trunk of *Abies borisii-regis* (Diamandis & Perlerou 1990) and from dead branches of *Fagus* (Diamandis 1992). The latter collection is accompanied by a description, but with no reference to the morphology of the cheilocystidia. Judging by the habitat, the first collection might be var. *subglobiger* and the second var. *applanatus* but the authors make no such distinction.

C. applanatus var. *subglobiger* is newly recorded for Greece.

Crepidotus cesatii var. *cesatii* (Rabenh.) Sacc. **Figs 3a–b; 9e**

Crepidotus cesatii (Rabenh.) Sacc., Michelia 1: 2 (1877); *Dochmiopus sphaerosporus* (Pat.) Pat., Hyménomyc. Eur.: 113 (1887); *Crepidotus sphaerosporus* (Pat.) J.E. Lange, Dansk Bot. Ark. 9 (6): 52 (1938); *Crepidotus cesatii* var. *sphaerosporus* (Pat.) A. Ortega & Buendía, Int. J. Myc. Lich. 4 (1–2): 96 (1989)

Pileus 3–22 mm, circular to semicircular or roundedly flabelliform, rarely somewhat lobed, convex to plano-convex, centrally to eccentrically or almost laterally attached to the substrate, margin incurved, becoming even only in fully mature specimens, surface dry, felty, pure white, remaining so in dried specimens or becoming pale cream. **Lamellae** whitish in young specimens, often with a pinkish tint, later cream, pinkish buff to pale cinnamon, never significantly darker, distant to subdistant, adnate, margin minutely fimbriate, remaining whitish. **Stipe** absent or rudimentary.

Basidiospores (5.5–) 6.5–8.0 (–9.0) × 4.5–7.0 µm, Q = (1.00–) 1.10–1.33 (–1.46), globose, subglobose to broadly ellipsoid, pale yellowish in KOH, finely echinulate (Figs 3a, 9e). **Basidia** 20–37 × 6–9 µm, cylindrical-clavate, 4-spored. **Cheilocystidia** 28–80 × 4–11 µm, diverticulate, clavate, cylindrical, irregularly cylindrical, fusiform, lageniform, usually branched, frequently multiply so, apices obtuse, hyaline, thin-walled (Fig. 3b). **Pileipellis** a trichodermium of loosely interwoven hyphae with transitions to a loose cutis, hyphae often coiled, 3–6 µm wide, hyaline, thin-walled. **Clamp connections** present in all tissues.

Habitat: Solitary or in small groups on dead or living branches of *Platanus orientalis* and, in one case, *Pinus nigra*.

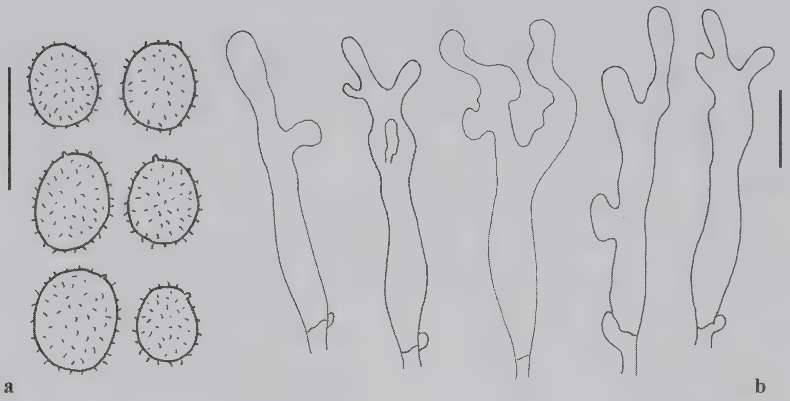


Fig. 3. *C. cesatii* var. *cesatii:* **a.** basidiopores, **b.** cheilocystidia. Scale bars = 10 µm.

Specimens examined — Mt. Zygourolivado, Anthochori, Karditsa, riparian vegetation, on a living branch of *Platanus orientalis,* 19 Sep. 1999, Delivorias, ATHU–M 5116; Mt. Katachloro, Kedros, Karditsa, riparian vegetation, on branches of *Pl. orientalis,* 13 Nov. 1999, Delivorias, ATHU–M 5118; Mt. Tymfristos, Raches Tymfristou, Evrytania, forest of *Pinus nigra* and *Abies borisii-regis,* on branches of *P. nigra,* 25 Oct. 2003, Delivorias, ATHU–M 5125; Mt. Tymfristos, Agios Nikolaos, Evrytania, forest of *Pl. orientalis, Quercus* sp. & *Castanea sativa,* on fallen twigs and branches of *Pl. orientalis,* 8 Nov. 2003, Gonou, ATHU–M 5109; Mt. Liakoura, Granitsa, Evrytania, forest of *A. borisii-regis* and *Pl. orientalis,* on dead twigs and branches of *Pl. orientalis,* 27 Sep. 2004, Delivorias, ATHU–M 5162; Mt. Tymfristos, Agios Nikolaos, Evrytania, mixed forest of *Pl. orientalis, Quercus frainetto, C. sativa* and *A. borisii-regis,* on branches of *Pl. orientalis,* 23 Oct. 2004, Gonou, ATHU–M 5173; Mt. Liakoura, Granitsa, Evrytania, forest road, on living branches of *Pl. orientalis,* 11 Nov. 2004, Delivorias, ATHU–M 5176; Mt. Liakoura, Granitsa, Evrytania, forest of *A. borisii-regis* and *Pl. orientalis,* on dead branches of *Pl. orientalis,* 11 Nov. 2004, Delivorias, ATHU–M 5179.

Remarks: The typical variety of *C. cesatii* is characterized by the distant lamellae, the globose to subglobose, finely echinulate basidiospores, the diverticulate cheilocystidia and the coiled hyphae of the pileipellis. The only other variety recognized by Senn-Irlet (1995), *C. cesatii* var. *subsphaerosporus* (J.E. Lange) Senn-Irlet, is reported to have broadly ellipsoid basidiospores, mostly straight hyphae on the pileipellis and to grow on branches of coniferous trees. Also, Watling & Gregory (1989), report that the latter variety lacks the characteristic pink tinge on the lamellae and has a darker spore print, although Senn-Irlet does not make such a reference. In all specimens examined in this work, at least a portion of the basidiospores were found to be broadly ellipsoid, and, in some specimens, these spores predominate. However, in all specimens the hyphae of the pileipellis were clearly coiled and the only collection made from coniferous trees does not seem to deviate microscopically from the remaining collections. As we have been unable to determine a correlation between the variation in shape of the basidiospores and either the structure of the pileipellis, the colour of the lamellae or the habitat, we have concluded that our findings consist of a single taxon, *C. cesatii* var. *cesatii,* in which the basidiospores may range from perfectly globose to broadly ellipsoid in their extreme variation, with a Q ratio reaching up to 1.46.

C. cesatii var. *cesatii* is newly recorded for Greece on twigs and branches of *Pinus nigra* and *Platanus orientalis,* the latter seeming a rather common substrate for the species in Greece, in contrast to the references for the distribution of the species in Europe (Senn-Irlet 1995).

Crepidotus variabilis (Pers.: Fr.) P. Kumm. **Figs 4a–b, 10g**

Crepidotus variabilis (Pers.: Fr.) P. Kumm., Führ. Pilzk.: 74 (1871)

Pileus 5–15 mm, circular to semicircular or roundedly flabelliform, often lobed, convex to plano-convex, centrally to eccentrically or almost laterally attached to the substrate, with incurved and later even margin, surface dry, felty to smooth, pure white to dirty white, remaining so in dried specimens. **Lamellae** whitish in young specimens, often with a pale pinkish tint, later clay buff and finally cinnamon brown, moderately crowded to crowded, emarginately adnate, margin minutely fimbriate, remaining whitish. **Stipe** absent or rudimentary.

Basidiospores (5.0–) 5.5–7.0 (–7.5) × (2.5–) 3.0–3.5 (–4.0) µm, Q = 1.57–2.17, short cylindric to elongate, oblong, pale yellowish in KOH, minutely but distinctly punctate-warty (Figs 4a, 10g). **Basidia** 20–25 × 5–7 µm, cylindrical-clavate, 4-spored. **Cheilocystidia** 22–53 × 6–11 µm, diverticulate, clavate, cylindrical, irregularly cylindrical, fusiform, mostly branched, often multiply branched, hyaline, thin-walled (Fig. 4b). **Pileipellis** a trichodermium of loosely interwoven hyphae, 2–5 µm wide, hyaline, thin-walled. **Clamp connections** present in all tissues.

Habitat: Solitary to gregarious on branches of *Quercus frainetto, Quercus coccifera* and *Cistus* sp.

Specimens examined — lake Plastira, Agios Athanasios, Karditsa, forest of *Quercus frainetto,* on fallen branches of *Q. frainetto,* 1 Nov. 1998, Delivorias, ATHU–M 5112; lake Plastira, Agios Athanasios, Karditsa, forest of *Q. frainetto,* on fallen branches of *Q. frainetto,* 1 Nov. 1998, Delivorias, ATHU–M 5113; lake Plastira, Agios Athanasios, Karditsa, forest of *Q. frainetto,* on fallen branches of *Q. frainetto,* 18 Sep. 1999, Delivorias, ATHU–M 5114, Domokos, Fthiotida, maquis vegetation, on branches of *Quercus coccifera,* 13 Nov. 1999, Delivorias, ATHU–M 5115; Mt. Ymittos, Attiki, maquis vegetation, on twigs of *Cistus* sp., 7 Dec. 2002, Dimitriadis, ATHU–M 4648.

Remarks: *C. variabilis* is characterized by the lobed pileal margin (not always distinct, however), the small-sized, cylindrical, punctate-warty basidiospores and the diverticulate cheilocystidia. The lobed pileal margin may be a good distinctive feature for macroscopical identification when clearly formed, but, as in all white species of *Crepidotus,* careful microscopical examination is essential for identification. The characteristic small-sized, cylindrical basidiospores provide a reliable distinguishing feature. *C. variabilis* var. *trichocystis* Hesler & A.H. Sm. is reported to have larger basidiospores and longer, narrowly cylindrical to narrowly lageniform cheilocystidia (Senn-Irlet 1995).

C. variabilis seems to be common in Greece, as it is reported a number of times in the literature (Maire & Politis 1940, as *Dochmiopus variabilis;* Minter 1988; Avtzis & Diamandis 1988; Konstantinidis 2002) and we have collected it a few times ourselves.

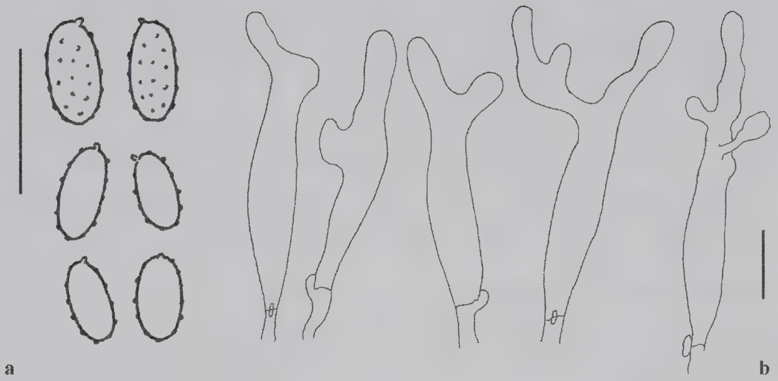


Fig. 4. *C. variabilis:* **a.** basidiospores, **b.** cheilocystidia. Scale bars = 10 µm.

It has also been recently collected on branches of *Alnus glutinosa* and *Quercus coccifera* (Dimou pers. com.). It is newly recorded for Greece on branches of *Quercus frainetto, Q. coccifera* and twigs of *Cistus* sp., the last two being representative plants of the maquis vegetation.

Section *Crepidotellae* Hesler & A.H. Sm., 1965

Subsection *Autochthoni* Senn-Irlet, 1995

Crepidotus autochthonus J.E. Lange **Figs 5a–b, 9f, 11c, e**

Crepidotus autochthonus J.E. Lange, Dansk bot. Ark. 4 (6): 51 (1938)

Pileus 10–40 mm, semicircular to flabelliform, convex to plano-convex, laterally or almost laterally attached to the substrate, with incurved, later even to undulating margin, surface dry, glabrous to minutely fibrillose-tomentose, dirty whitish, cream to yellowish buff. **Lamellae** whitish in young specimens, then spotted brownish and finally uniformly cinnamon brown to fulvous, crowded, emarginately adnate, margin even. **Stipe** absent or rudimentary. **Spore print** yellowish brown to umber.

Basidiospores 7.0–9.0 × 5.0–6.0 µm, Q = 1.27–1.64, ellipsoid, amygdaliform or lemoniform in side view, with a more or less acute apex, smooth, thick-walled, occasionally wall thinning at acute apex, yellowish to yellowish brown in KOH, usually with a large oil drop (Figs 5a, 9f). **Basidia** 25–30 × 7–9 µm, cylindrical-clavate, with 4 sterigmata. **Cheilocystidia** 17–32 × 7–13 µm, cylindrical, clavate, some subcapitate, short lageniform, not branched, rarely septate, thin to thick-walled (Figs 5b, 11c). Basidioles and cheilocystidia sometimes with yellow-golden, smooth content (Fig. 11e). **Pileipellis** a cutis of hyaline hyphae, 3–5 µm wide, some ascending. **Pileal trama** without gelatinous layer. **Lamellar trama** with few secretory hyphae, usually hyaline, seldom golden-yellow. **Clamp connections** present in all tissues.

Habitat: Gregarious or in small groups on ground, in forest of *Quercus frainetto* or mixed *Q. frainetto* and *Abies borisii-regis.*

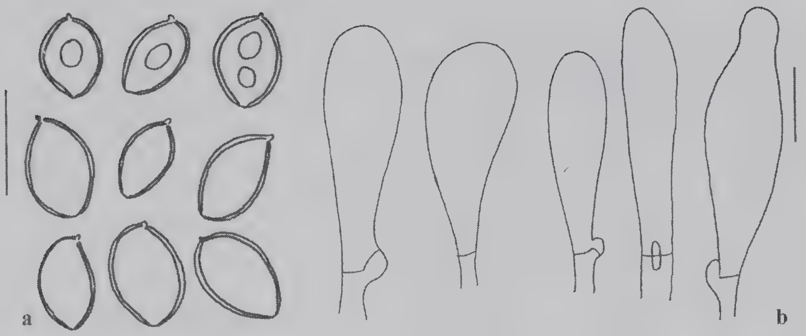


Fig. 5. *C. autochthonus:* **a.** basidiospores, **b.** cheilocystidia. Scale bars = 10 µm.

Specimens examined — Lake Plastira, Kryoneri, Karditsa, clearing of forest of *Quercus frainetto,* on ground, 14 Oct. 2000, Delivorias, ATHU–M 5121; lake Plastira, Kastania, Karditsa, mixed forest of *Q. frainetto* and *Abies borisii-regis,* on ground, 7 Sep. 2002, Delivorias, ATHU–M 5124.

Remarks: *C. autochthonus* is rather similar-looking macroscopically to *C. mollis* and has practically identical basidiospores, which nevertheless can be distinguished by their acute apex, without the characteristic wall curving or apical depression of *C. mollis* spores. It is also easily identifiable by the terrestrial habit, the lack of a gelatinous layer in the pileal trama, the shape of the cheilocystidia and the presence of clamp-connections. According to Senn-Irlet (1995), it is the only terrestrial species of *Crepidotus* in Europe.

C. autochthonus is newly recorded from Greece and is reported for the first time in Europe in forests of *Quercus* spp.

Subsection *Pleurotellus* (Fayod) Senn-Irlet, 1995

Crepidotus epibryus (Fr.: Fr.) Quél. **Figs 6a–b; 10i; 11f**

Crepidotus epibryus (Fr.: Fr.) Quél., Mém. Soc. Emul. Montbéliard, sér. 2, 5: 138 (1872);
misdet.: *Crepidotus perpusillus* (Lumn.: Fr.) Maire, Fungi Catal. II: 102 (1937)

Pileus up to 10 mm, rounded flabelliform or campanulate when young, circular with age, spreading out on the substrate, becoming almost resupinate, with white tomentum around the margin where attached, sessile, surface tomentose, white, even when dried. **Lamellae** rather distant to moderately crowded, adnexed, whitish to pale ochraceous in fresh specimens, remaining so or darkening to fulvous in dried ones, margin concolorous, even to slightly uneven, often browning at places, especially when dried. **Flesh** very thin, white. **Stipe** absent.

Basidiospores 6.0–9.0 × 2.5–3.0 µm, cylindrical, somewhat fusoid to narrowly amygdaliform or pip-shaped, some slightly curved, smooth, hyaline or pale yellow in KOH, yellow in the commonly formed masses (of two, four or more) (Figs 6a, 10i). **Basidia** 15–20 × 5–6 µm, clavate, 4-spored, usually hyaline; some disintegrating basidioles and basidia with granular, golden brown content, larger in size (Fig. 11f). **Cheilocystidia** up to 50 × 7 μm, cylindrical to narrowly lageniform, flexuous, sometimes with strongly curved or whirled apex, rarely branched, often difficult to be observed (Fig. 6b). Parts of the hymenium covered with golden-brown granular material. **Pileipellis** a transition between a cutis of interwoven hyphae and a trichodermium of erect, straight, filiform, hyaline hyphae. **Clamp connections** absent in all tissues.

Habitat: Solitary on stalks and laminas of fallen leaves of *Castanea sativa.*

> **Specimens examined**: Mt. Tymfristos, Agios Nikolaos, Evrytania, mixed forest of *Castanea sativa, Abies borisii-regis, Quercus* sp. and *Corylus avelana,* on fallen leaves of *C. sativa,* 14 Nov. 1998, Gonou, ATHU–M 5106.

Remarks: Distinctive microscopical characters of *C. epibryus* are the size and shape of the basidiospores as well as the narrowly lageniform, flexuous and/or curled at the apex cheilocystidia. The cheilocystidia were scarce and difficult to find, possibly because the lamellar edge had been injured. The golden-brown material covering parts of the hymenium is probably the result of the excretion of necropigments from the concolorous disintegrating basidia and basidioles and corresponds macroscopically to the browning spots of the lamellae. Similar pigmented and amorphous aggregations on the hymenium are reported from species of *Lyophyllum* as well as pigmented basidia from species of *Inocybe, Cortinarius* and *Pholiota* (Clémençon 1997).

C. epibryus has been twice reported from Greece, on leaves of *Fagus sylvatica* (as *Crepidotus perpusillus,* Maire & Politis 1940) and on leaves of *Quercus cerris* (Polemis et al. 2002). Our collection is the first recording of *C. epibryus* on leaves of *Castanea sativa* in Greece and one of the very few in Europe (Senn-Irlet 1995).

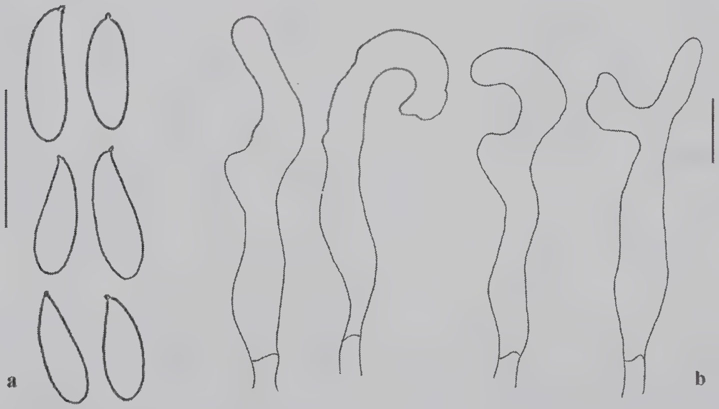


Fig. 6. *C. epibryus:* **a.** basidiospores, **b.** cheilocystidia. Scale bars = 10 μm.

Subsection *Fibulatini* Singer, 1947

Crepidotus lundellii Pilát **Figs 7a–b; 10a, d**

Crepidotus lundellii Pilát, Fungi Exsiccati Suecici fasc. V-VI: 10 (1936)

Pileus 3–20 mm, circular to semicircular or roundedly flabelliform, convex to plano-convex, eccentrically to almost laterally attached to the substrate, with incurved and later even margin, surface dry, felty, pure white to pale cream. **Lamellae** whitish in young specimens, then clay buff and finally cinnamon brown to fulvous, moderately crowded to crowded, emarginately adnate, margin minutely fimbriate, remaining whitish. **Stipe** absent or rudimentary.

Basidiospores 6.5–8.5 (–9.0) × 4.5–5.5 (–6.0) µm, Q = 1.40–1.80, in frontal view broadly ovoid to ellipsoid, in side view ellipsoid to slightly amygdaliform, yellowish in KOH, wall slightly roughened, almost smooth, frequently seemingly smooth even under oil immersion but never actually completely smooth (Figs 7a, 10a). **Basidia** 21–30 × 6–8 µm, cylindrical-clavate, 4-spored. **Cheilocystidia** 32–56 × 7–12 µm, clavate, cylindrical, fusiform, with an obtuse, sometimes subcapitate apex, rarely branched, hyaline, thin-walled (Figs 7b, 10d). **Pileipellis** a trichodermium of loosely interwoven hyphae 3–5 µm wide, hyaline, thin-walled. **Clamp connections** present in all tissues.

Habitat: Gregarious on fallen branches of *Platanus orientalis.*

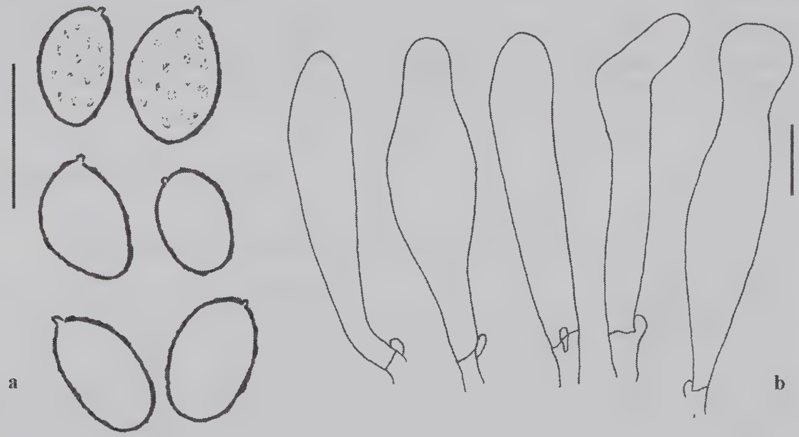


Fig. 7. *C. lundellii:* **a.** basidiospores, **b.** cheilocystidia. Scale bars = 10 µm.

Specimens examined — Mt. Katachloro, Kedros, Karditsa, riparian vegetation, on fallen branches of *Platanus orientalis,* 13 Nov. 1999, Delivorias, ATHU–M 5119; Mt. Tymfristos, Agios Nikolaos, Evrytania, mixed forest of *P. orientalis, Quercus frainetto, Castanea sativa* and *Abies borisii-regis,* on branches of *P. orientalis*, 23 Oct. 2004, Gonou, ATHU–M 5171.

Remarks: *C. lundellii* is characterized by the unique ornamentation of the basidiospores, consisting of very low warts and ridges. The spores seem smooth in low magnification and the ornamentation is revealed only under oil immersion. The basidiospores are ellipsoid to slightly amygdaliform and faint yellowish in KOH. In the macroscopically similar *C. subverrucisporus,* the spore ornamentation is much more distinct, the spore colour is darker and the cheilocystidia are more consistently narrowly lageniform (apically tapered) (Senn-Irlet 1995; Bandala et al. 1999).

C. lundellii is newly recorded for Greece and is reported for the first time on *Platanus* in Europe (Senn-Irlet 1995). It has also been recently collected on branches of *Alnus glutinosa* (Dimou pers. com.).

Crepidotus luteolus (Lambotte) Sacc. **Figs 8a–b; 10b, e**

Crepidotus luteolus (Lambotte) Sacc., Syll. Fung. (Abellini) 5: 888 (1887)

Pileus 2-15 mm, sessile, young ungulate, campanulate, later convex to plano-convex, flabelliform, reniform or semicircular when seen from above, laterally or dorsally attached, often spread out over the substrate, almost resupinate, margin even, straight, remaining so or becoming undate, lobate, white-yellowish to yellowish-cream when wet, straw to buff when dried, surface first tomentose-hirsute with a smooth margin, later smooth throughout or hirsute only near the point of attachment, usually with a rich tomentum on the substrate. **Lamellae** adnexed to narrowly adnate, moderately crowded, whitish or pale yellowish at first, later buff to buffish brown, fulvous when dried, margin whitish, fimbriate when young, concolorous and almost smooth when mature. **Flesh** white. **Stipe** absent or rarely observed in very young fruit bodies.

Basidiospores 8.0–9.5 (–10.5) × 4.5–5.5 (–6.0) µm, Q = 1.55–2.10, ellipsoid in frontal view, ellipsoid to usually amygdaliform in side view, yellowish brown in KOH, minutely roughened (Figs 8a, 10b). **Basidia** 20–30 × 7–9 µm, clavate, usually strongly granular, 4-spored. **Cheilocystidia** 45–60 × 5–8 µm, cylindrical to narrowly lageniform, strongly flexuous, often branched or rarely angular, hyaline, thin walled (Figs 8b, 10e). **Lamellar edge** in some sections somewhat gelatinous, holding cheilocystidia rather packed. **Pileipellis** either a cutis of interwoven hyphae with transitions to a trichodermium bearing bundles of exerted hyphal ends, or a real trichodermium with erect hyphae, hyphae straight, flexuous or winding, thin to slightly thick walled, hyaline or pale yellow, some of the latter slightly encrusted. **Clamp connections** present in all tissues.

Habitat: Solitary or in small groups, rather gregarious, on fallen twigs of *Platanus orientalis.*

Specimens examined — Mt. Tymfristos, Agios Nikolaos, Evrytania, mixed deciduous forest of *Platanus orientalis, Quercus* sp. and *Castanea sativa,* on fallen twigs of *P. orientalis,* 8 Nov. 2003, Gonou, ATHU–M 5110.

Remarks: *C. luteolus* is characterized macroscopically by the yellowing colors of the basidiocarps and microscopically by the faintly ornamented, amygdaliform, relatively long and narrow basidiospores as well as the polymorphic, flexuous, frequently branched cheilocystidia. Our specimens of *C. subverrucisporus* exhibit the same yellowing colors when fresh and could be confused macroscopically with those of *C. luteolus.* The two species can be distinguished microscopically by their basidiospores and cheilocystidia. The basidiospores of *C. subverrucisporus* differ in being more ornamented and ellipsoid (broader) rather than amygdaliform in side view, while the cheilocystidia are less flexuous and branched.

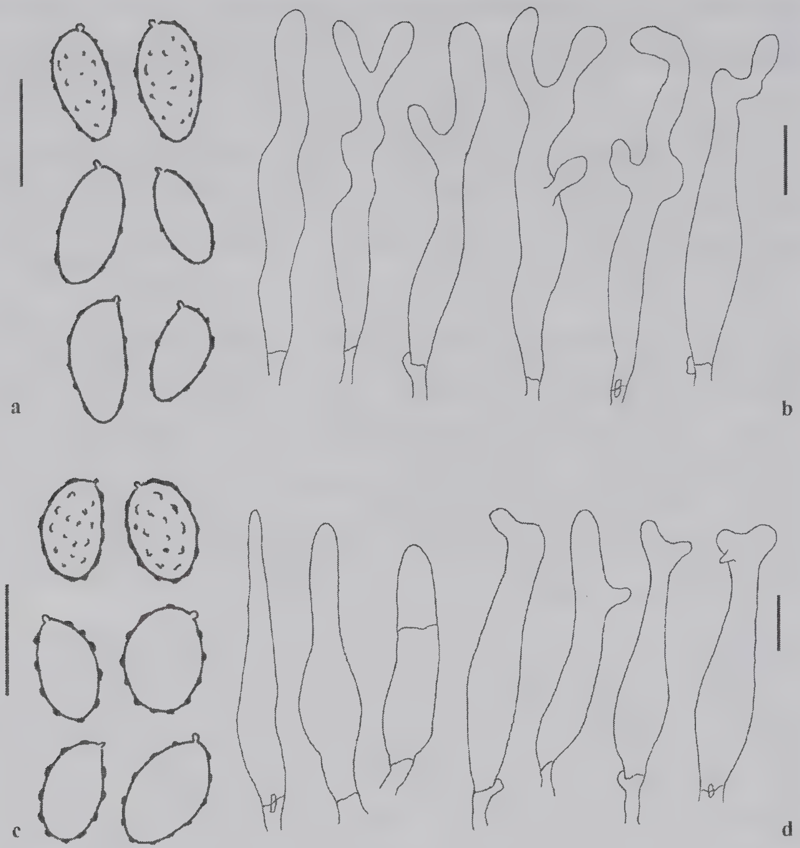


Fig. 8. a–b. *C. luteolus:* **a.** basidiospores, **b.** cheilocystidia; **c–d.** *C. subverrucisporus:* **c.** basidiospores, **d.** cheilocystidia. Scale bars = 10 μm.

It is worth mentioning that besides *C. luteolus*, specimens of *C. cesatii* were collected on the same day from the same locality, on nearby fallen twigs of *Platanus orientalis*. Basidiocarps of *C. luteolus* exhibited a pale yellow color on the pileus in contrast to the whitish color of *C. cesatii.*

C. luteolus is newly recorded from Greece and is reported for the first time on *Platanus* in Europe. It has also been recently collected on *Nerium oleander* (Dimou pers. com.)

Crepidotus subverrucisporus Pilát **Figs 8c–d; 10c, f; 11a**

Crepidotus subverrucisporus Pilát, Studia Botanica Čechoslavaca 10: 151 (1949)

Pileus 3–10 mm, circular to semicircular or roundedly flabelliform, convex to plano-convex, eccentrically to almost laterally attached to the substrate, where usually with a whitish or yellowish tomentum, margin incurved and later even, surface dry, felty, white or dirty whitish to pale yellowish cream. **Lamellae** whitish in young specimens, later buff and finally cinnamon brown to fulvous, moderately crowded, emarginately adnate, margin minutely fimbriate, remaining whitish. **Stipe** absent.

Basidiospores (7.0–) 7.5–10.0 (–11.0) × (4.5–) 5.0–6.0 (–7.0) μm, Q = 1.45–1.78, ovoid to ellipsoid, slightly amygdaliform in side view, yellowish brown in KOH, minutely but distinctly rugulose (Figs 8c, 10c). **Basidia** 20–25 × 7–8 μm, cylindrical-clavate, 4-spored, some 2-spored. **Cheilocystidia** 28–65 × 4–10 × 3–5 μm, cylindrical, broadly lageniform to narrowly, elongate lageniform, often angular or sometimes branched towards the apex or apex subcapitate, sometimes septate at upper 1/3, hyaline, thin-walled (Figs 8d, 10f, 11a). **Pleurocystidia** absent but seldom lageniform pleurocystidioid bodies present. Basidioles and pleurocystidioid bodies sometimes with golden-yellow content. **Pileipellis** a trichodermium with transitions to a cutis, hyphae loosely interwoven, 3–6 μm wide, hyaline, thin-walled. **Clamp connections** present in all tissues.

Habitat: Gregarious on fallen branches of *Platanus orientalis.*

> **Specimens examined** — Mt. Katachloro, Kedros, Karditsa, riparian vegetation, on fallen branches of *Platanus orientalis,* 13 Nov. 1999, Delivorias, ATHU–M 5120; Mt. Tymfristos, Agios Nikolaos, Evrytania, mixed forest of *P. orientalis, Quercus frainetto, Castanea sativa* and *Abies borisii-regis,* on mossy branches of *P. orientalis,* 23 Oct. 2004, Gonou, ATHU–M 5172.

Remarks: The fresh fruit bodies from both collections exhibited a pale yellowish color in the pileus which was retained in the exsiccata. *C. subverrucisporus* is characterized by the minutely but distinctly rugulose, slightly amygdaliform, moderately dark coloured basidiospores and the rather simple lageniform cheilocystidia. The cheilocystidia are reported to be generally unbranched (Watling & Gregory 1989; Senn-Irlet 1995; Breitenbach & Kränzlin 2000). Type studies performed by Senn-Irlet (1993) showed that the cheilocystidia in the holotype (found on *Robinia pseudoacacia*) are often septate (also observed by Bandala et al. 1999) and sometimes branched. The same observation was made by Senn-Irlet (1995) on material collected from Italy, also on *Robinia.* We have encountered in our specimens both septate and branched at the tips cystidia.

We have also occasionally observed pleurocystidioid-like bodies, some with a golden-yellow content, but they are probably of no taxonomic significance. Bandala et al. (1999) also report sterile basidiole-like elements rarely present on the sides of the lamellae and consider them probably to be abnormal basidia or basidioles.

C. subverrucisporus is newly recorded from Greece and is reported for the first time on *Platanus* in Europe.

Discussion

With few exceptions, such as *C. calolepis* or *C. cinnabarinus,* the species of *Crepidotus* are more or less macroscopically similar, i.e. whitish, small to medium sized basidiocarps, with whitish to pale brownish lamellae and without a distinctive smell or taste. Hence, careful microscopic examination is absolutely essential for determination at a species level. The most important microscopic features from a taxonomical standpoint are the morphology of basidiospores, cheilocystidia and, to a lesser degree, pileipellis, and the presence or absence of clamp connections (Nordstein 1990; Senn-Irlet 1995; Bandala & Montoya 2004). In few species other features are also of taxonomical importance, such as the presence of a gelatinous layer in the pileal trama in *C. calolepis* and *C. mollis,* the distant, pinkish lamellae in *C. cesatii,* or some striking habitat preferences, such as the growth on stems, leaves or litter in *C. epibryus,* or directly on soil in *C. autochthonus.* In general, however, habitat preferences are an indicative and not a decisive taxonomical feature. *C. calolepis* and *C. mollis* are considered by most authors to grow exclusively on deciduous trees, but we have collected both species on conifers in more than a few occasions. *C. cesatii* var. *cesatii* is considered to prefer deciduous trees whereas var. *subsphaerosporus* coniferous trees, but the decisive distinctive feature between the two varieties is the shape of the basidiospores and not the habitat. We have collected specimens of *C. cesatii* on both deciduous and coniferous trees, but without any notable microscopic differences between them.

We noted many deviant features in our specimens, such as a callus-like structure at the apex of the basidiospores in *C. calolepis* and *C. mollis,* pleurocystidioid-like bodies in *C. calolepis, C. applanatus* var. *subglobiger* and *C. subverrucisporus,* septate cheilocystidia in *C. mollis, C. applanatus* var. *subglobiger, C. autochthonus* and *C. subverrucisporus,* thick-walled cheilocystidia in *C. applanatus* var. *subglobiger,* basidia, basidioles and cheilocystidia with pigmented content in *C. mollis, C. autochthonus* and *C. subverrucisporus,* amorphous material on the hymenium in *C. epibryus,* gelatinized lamellar trama in *C. calolepis* and *C. mollis,* and secretory hyphae in many species. The presence of the callus-like structure in *C. calolepis* and *C. mollis,* although sporadic, is constant and should be considered characteristic for the two species. Singer (1973) also mentioned such features. All the other features have an inconstant appearance and are due most probably to environmental conditions or different stages of maturity, and therefore no taxonomic value can be attributed to them.

The determination of the diversity and distribution of *Crepidotus* species is not an easy task, as they usually form small-sized basidiocarps that may be overlooked. Prior to this work, 7 taxa of *Crepidotus* were reported from Greece: *C. applanatus* (Diamandis & Perlerou 1990; Diamandis 1992), *C. cesatii* (Maire & Politis 1940, as *Dochmiopus sphaerosporus,* Dimou et al. 2002a, as *Crepidotus sphaerosporus*), *C. calolepis* (Maire & Politis 1940), *C. cinnabarinus* (Dimou et al. 2002b), *C. epibryus* (Maire & Politis 1940, as *Crepidotus perpusillus;* Polemis et al. 2002), *C. mollis* (Diapoulis 1939; Maire & Politis 1940; Pantidou 1991; Zervakis et al. 1998; Dimou et al. 2002a) and *C. variabilis* (Maire & Politis 1940; Minter 1988; Avtzis & Diamandis 1988; Diamandis 1992; Konstantinidis 2002). Most of these taxa were reported only once or twice, with the exceptions of the seemingly common *C. variabilis* and *C. mollis.* Concerning the latter, as stated in this work, it is possible that some collections attributed to *C. mollis* may in fact represent collections of *C. calolepis.* Some older collections (Diapoulis 1939; Maire & Politis 1940) cannot be accounted for and probably have not survived to this day. We have examined collection ATHU–M 1071 identified as *C. mollis* (Pantidou 1991) and have attributed it to *C. calolepis,* and have also cross-examined two specimens of *C. calolepis* and one of *C. mollis,* kindly provided to us by Dimou. We are convinced that *C. calolepis* is much more common in Greece than *C. mollis.*

Crepidotus species never form the dominant element in any European vegetation unit (Senn-Irlet 1995), and this obviously applies for Greece as well. Most of the gathered collections consist of rather few individuals. Few of the collected species were found to grow gregariously, i.e. *C. calolepis, C. mollis, C. autochthonus* and, to a lesser degree, *C. luteolus* and *C. subverrucisporus.* However, in some occasions we collected more than a few different species from the same location. Seven taxa: *C. calolepis, C. mollis, C. lundellii, C. subverrucisporus, C. cesatii* var. *cesatii, C. luteolus* and *C. epibryus,* were collected from a small area on Mt. Tymfristos (Agios Nikolaos), the first five on the same day. In addition, *C. calolepis* and *C. cesatii* were collected in two successive years from that area. Three of the similar-looking white species, *C. cesatii, C. lundellii* and *C. subverrucisporus,* were collected on the same day from the same location (Mt. Katachloro). In one case, basidiocarps of two species, *C. cesatii* and *C. calolepis,* were found on nearby branches of the same *Platanus orientalis,* a few meters apart from a second *Platanus* colonized by *C. mollis* (Mt. Liakoura). We have not, however, encountered more than one species on the same branch. Senn-Irlet (1995) states that in no case had she encountered basidiocarps of more than one *Crepidotus* species on the same substrate.

Floristic studies in other countries of the Mediterranean region reveal a similar diversity of *Crepidotus* species as in Greece. Senn-Irlet (1995) provides data for most countries surrounding the Mediterranean, such as Bulgaria, Jugoslavia, Turkey, Italy, France and Spain. A recent checklist of *Crepidotus* in Croatia includes eight species in total, all of which have also been found in Greece (Tkalčec & Mešić 2003). The same applies for the seven species of *Crepidotus* reported from Turkey (Selsi & Denchev 2005). The diversity of *Crepidotus* in Spain (Ortega & Buendía 1989) is also similar to that of Greece. Lonati (2000) presented a paper concerning the diversity of *Crepidotus* in the Mediterranean area, in which he reports sixteen species (twelve, if synonymy is considered) collected from various provinces of Italy. Malençon & Bertault (1975) report eight species from Morocco, Algeria and Tunisia.

In conclusion, the biodiversity of *Crepidotus* in Greece includes, to date, 12 taxa in total. A survey of all species recorded in Greece is given in Table 1, where both published and unpublished data are included. Five taxa are newly recorded from Greece: *C. applanatus* var. *subglobiger, C. autochthonus, C. lundellii, C. luteolus* and *C. subverrucisporus.* Other five taxa are recorded on new substrates for Greece: *C. calolepis* on trunks and branches of *Abies cephalonica, Abies borisii-regis* and *Pinus nigra, C. cesatii* var. *cesatii* on twigs and branches of *Platanus orientalis* and *Pinus nigra, C. epibryus* on leaves of *Castanea sativa,* and *C. variabilis* on twigs and branches of *Cistus* sp., *Quercus frainetto* and *Q. coccifera.* Moreover, *C. calolepis* and *C. mollis* are encountered for the first time in Europe on conifers, *C. calolepis, C. lundellii, C. luteolus* and *C. subverrucisporus* on *Platanus, C. autochthonus* in *Quercus* forests and *C. variabilis* on *Cistus.*

Acknowledgments

We wish to thank Drs. Beatrice Senn-Irlet and Victor Bandala for their thorough revision of the paper, valuable comments and constructive correspondence and Dr. Shaun Pennycook for his nomenclatural review. We thank D. Dimou and S. Diamandis for giving us useful information from their data on the distribution of *Crepidotus* species in Greece and especially the former for the provision of dried specimens for microscopic examination.

Table 1. A survey of *Crepidotus* species recorded from Greece.
(*) = first record of species and/or its substrate for Greece, (◆) = first record of substrate for Europe.

Species	Substrate	Habitat	Publication
C. applanatus	fallen branches of *Fagus moesiaca*	forest of *F. moesiaca*	Diamandis 1992
	fallen trunks of *Abies borisii-regis*	mixed forest of *A. borisii-regis* and *Pinus nigra*	Diamandis & Perlerou 1990
C. applanatus var. ***subglobiger***	fallen trunk of *Picea abies*	forest of *Picea abies*	this work (*)
C. autochthonus	soil	clearing of forest of *Quercus frainetto* (◆)	this work (*)
	soil	mixed forest of *Q. frainetto* & *A. borisii-regis* (◆)	this work (*)
C. calolepis	fallen twigs of *Pinus* sp.	forest of *Pinus* sp.	this work
	fallen branches of conifers	mixed forest of *P. nigra* & *Abies cephalonica*	this work
	stump of *A. cephalonica* (◆)	mixed forest of *A. cephalonica* & *P. nigra*	this work
	fallen twigs of *Platanus orientalis* (◆)	mixed forest of *A. cephalonica* & *Pl. orientalis* (sporadically)	this work
	fallen branches of conifers	mixed forest of *P. nigra* & *Abies* sp.	this work
	fallen branches of hardwoods	mixed forest of *Castanea sativa* & *Quercus* sp.	this work
	living trunk of *Pl. orientalis* (◆)	riparian vegetation	this work
	base of trunk of *Pl. orientalis* (◆)	mixed forest of *Pl. orientalis, Quercus* sp. & *C. sativa*	this work
	fallen twigs of *Pl. orientalis* (◆)		this work
	fallen twigs of *A. borisii-regis* (◆)	mixed forest of *A. borisii-regis* & *Pl. orientalis* (sporadically)	this work
	branches of *Pl. orientalis* (◆)	mixed forest of *Pl. orientalis, Q. frainetto, C. sativa* and *A. borisii-regis*	this work
	fallen branches of *P. nigra* (◆)	forest of *P. nigra*	this work
	living branches of *Pl. orientalis* (◆)	forest road	this work
	fallen trunk of *Pl. orientalis* (◆)	mixed forest of *A. borisii-regis* and *Pl. orientalis*	this work
	dead trunk of *Platanus* sp.		Maire & Politis 1940
	stump of *Pinus halepensis*		Maire & Politis 1940
	wood of *Pl. orientalis* (◆)		as *C. mollis;* Pantidou 1991
	wood of *Fagus sylvatica*		as *C. mollis;* Zervakis et al. 1998; Dimou et al. 2002a
			as *mollis* var. *calolepis;* pers. com., Konstantinidis
	fallen branches of *Alnus glutinosa*		as *C. mollis;* pers. com., Dimou
	dead trunk of *Pl. orientalis* (◆)		as *C. mollis* var. *calolepis;* pers. com., Dimou
C. cesatii var. ***cesatii***	living trunk of *Pl. orientalis* (*)	riparian vegetation	this work
	fallen twigs of *Pl. orientalis* (*)	riparian vegetation	this work
	fallen twigs of *Pl. orientalis* (*)	mixed forest of *Pl. orientalis, Quercus* sp. & *C. sativa*	this work
	twigs of *P. nigra* (*)	mixed forest of *P. nigra* & *A. borisii-regis*	this work
	dead twigs and branches of *Pl. orientalis* (*)	mixed forest of *A. borisii-regis* and *Pl. orientalis*	this work
	branches of *Pl. orientalis* (*)	mixed forest of *Pl. orientalis, Q. frainetto, C. sativa* and *A. borisii-regis*	this work
	living branches of *Pl. orientalis* (*)	forest road	this work
	dead branches of *Pl. orientalis* (*)	mixed forest of *A. borisii-regis* and *Pl. orientalis*	this work
	fallen twigs & leaves of *Fagus* sp.	mixed forest of *P. nigra* & *A. borisi-regis*	as *Dochmiopus sphaerosporus;* Maire & Politis 1940
	wood of *F. sylvatica*		as *C. sphaerosporus;* Dimou et al. 2002a
	wood of *Pl. orientalis* (*)		pers. com., Dimou
	hardwoods		pers. com., Dimou

Species	Substrate	Habitat	Publication
C. cinnabarinus	branches of *Alnus glutinosa*		Dimou et al. 2002b
	wood of *Pl. orientalis*	riparian vegetation	pers. com., Diamandis
C. epibryus	fallen leaves of *C. sativa* (*)	mixed forest of *Pl. orientalis, Quercus* sp. & *C. sativa*	this work
	fallen leaves of *Fagus* sp.		as *C. perpusillus;* Maire & Politis 1940
			Polemis et al 2002
C. lundellii	twigs of *Pl. orientalis* (◆)	riparian vegetation	this work (*)
	branches of *Pl. orientalis* (◆)	mixed forest of *Pl. orientalis, Q. frainetto, C. sativa* and *A. borisii-regis*	this work (*)
	branches of *Alnus glutinosa*		pers. com., Dimou
C. luteolus	twigs of *Pl. orientalis* (◆)	mixed forest of *Pl. orientalis, Quercus* sp. & *C. sativa*	this work (*)
	Nerium oleander		pers. com., Dimou
C. mollis	fallen trunk of *A. borisii-regis* (◆)	forest of *A. borisii-regis*	this work
	branches of *Pl. orientalis*	mixed forest of *Pl. orientalis, Q. frainetto, C. sativa* and *A. borisii-regis*	this work
	living trunk and branches of *Pl. orientalis*	mixed forest of *A. borisii-regis* and *Pl. orientalis*	this work
	dead branches of *Pl. orientalis*	mixed forest of *A. borisii-regis* and *Pl. orientalis*	this work
	old trunks of *Quercus* sp.		Diapoulis 1939
	stump of *Platanus* sp		Maire & Politis 1940
			pers. com., Konstantinidis
	hardwoods		pers. com., Dimou
C. subverrucisporus	fallen trunk of *Pl. orientalis*(◆)	riparian vegetation	this work (*)
	mossy branches of *Pl. orientalis* (◆)	mixed forest of *Pl. orientalis, Q. frainetto, C. sativa* and *A. borisii-regis*	this work (*)
C. variabilis	twigs of *Q. frainetto* (*)	forest of *Q. frainetto*	this work
	twigs of *Quercus coccifera* (*)	maquis vegetation	this work
	twigs of *Cistus* sp. (◆)	maquis vegetation	this work
	dead twigs of *Abies* sp.		as *Dochmiopus variabilis;* Maire & Politis 1940
	fallen branches of *Fagus moesiaca*		Avtzis & Diamandis 1988; Diamandis 1992
	dead twig		Minter 1988
	wood of deciduous trees & shrubs		Konstantinidis 2002
	branches of *Alnus glutinosa*		pers. com., Dimou
	branches of *Q. coccifera*		pers. com., Dimou
	wood of *C. sativa*	forest of *C. sativa*	pers. com., Diamandis
	wood of *A. borisii-regis*	mixed forest of *A. borisii-regis* and *P. nigra*	pers. com., Diamandis
	wood of *C. sativa*	forest of *C. sativa*	pers. com., Diamandis
	wood of *Q. frainetto* (*)	forest of *Q. frainetto*	pers. com., Diamandis
	wood of *Quercus* sp.	boundaries of plantation of *P. nigra*	pers. com., Diamandis
	wood of *A. borisii-regis*	forest of *A. borisii-regis*	pers. com., Diamandis

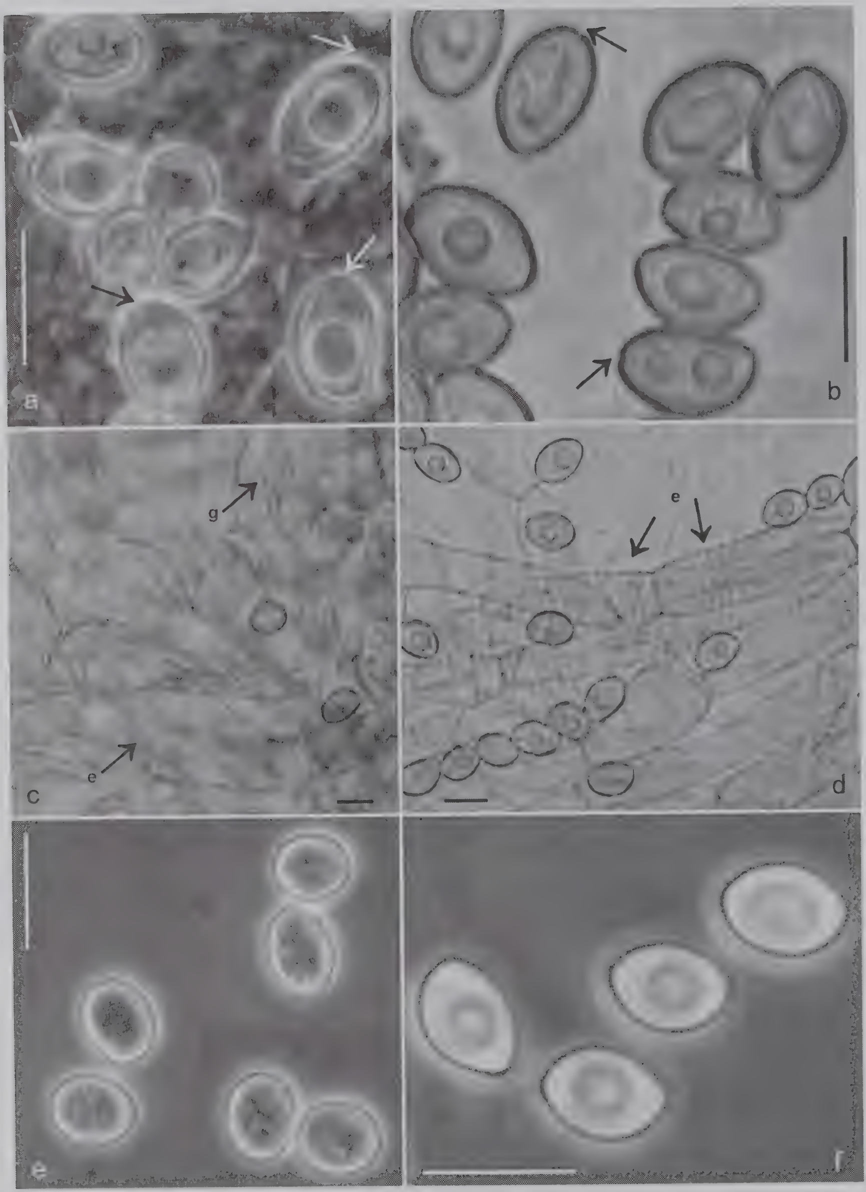


Fig. 9. a–b. Thick-walled basidiospores with apical wall curving and small depression (arrows); **c.** pale yellow hyphae of the pileipellis with granular (g) or minutely encrusting (e) pigment; **d.** yellow-brown hyphae of the pileipellis with strongly encrusting zebra-like pigment (e): **a, c.** *C. mollis* (ATHU–M 5123); **b, d.** *C. calolepis* (Dimou 731). **e–f.** Basidiospores: **e.** *C. cesatii* var. *cesatii* (ATHU–M 5109, 5125); **f.** *C. autochthonus* (ATHU–M 5121). Scale bars = 10 μm.

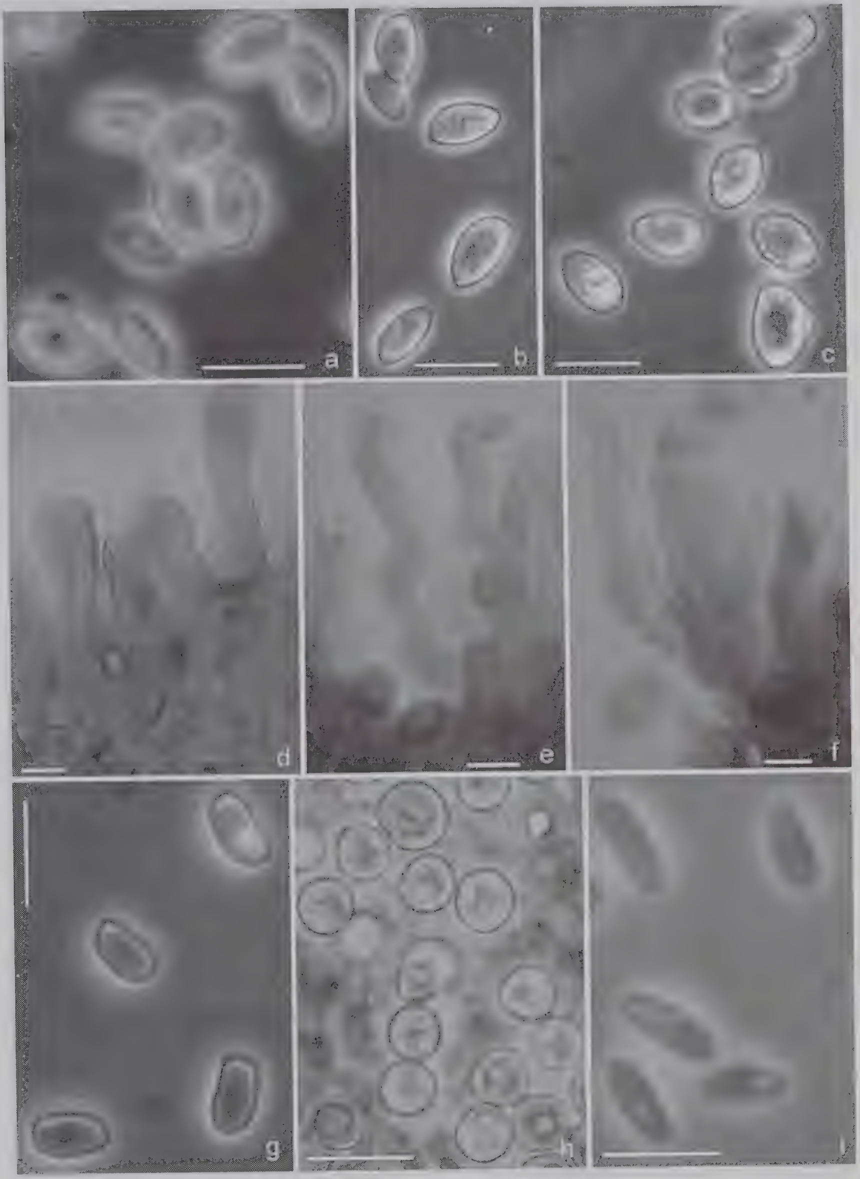


Fig. 10. a–c. Basidiospores; **d–f.** Cheilocystidia: **a, d.** *C. lundellii* (ATHU-M 5119); **b, e.** *C. luteolus* (ATHU-M 5110); **c, f.** *C. subverrucisporus* (ATHU-M 5120). **g–h.** Basidiospores: **g.** *C. variabilis* (ATHU-M 5112); **h.** *C. applanatus* var. *subglobiger* (ATHU-M 5332); **i.** *C. epibryus* (ATHU-M 5106). Scale bars = 10 μm.

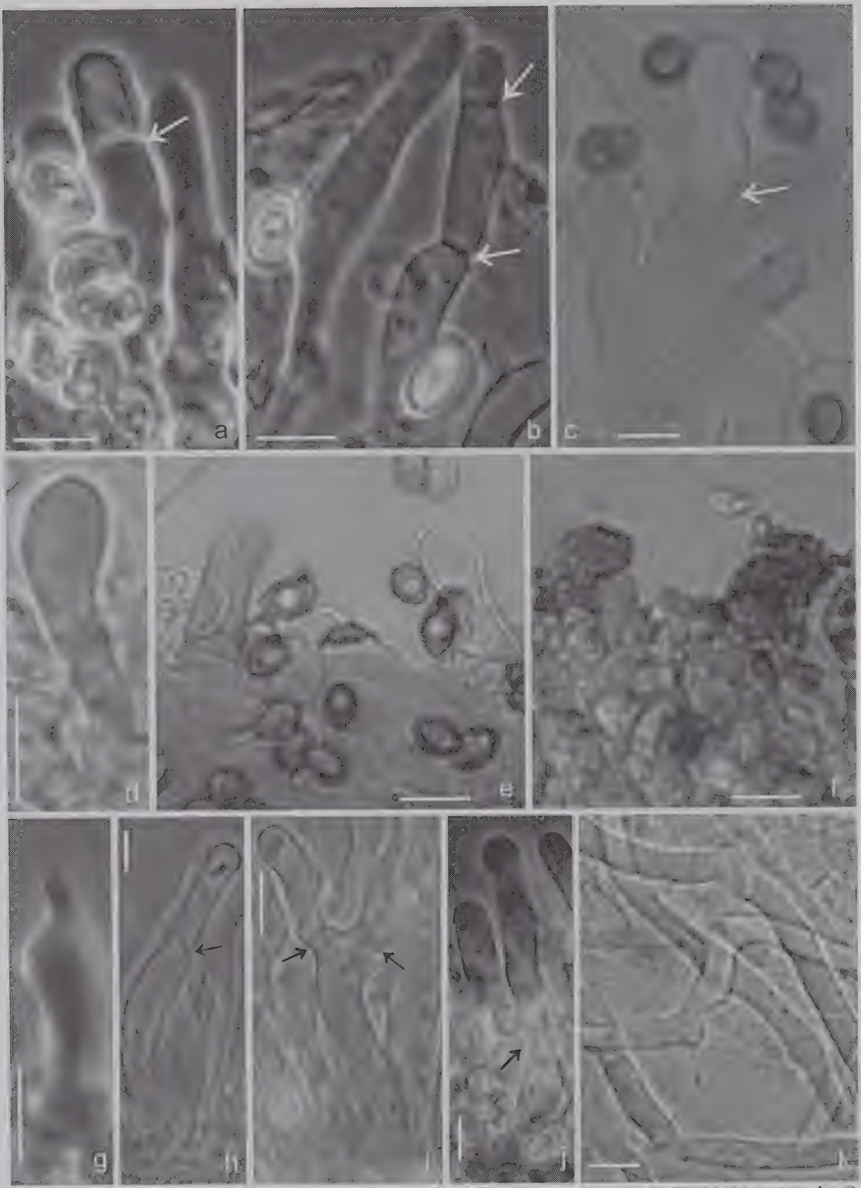


Fig. 11. a–c. Cheilocystidia with septae (arrows): **a.** *C. subverrucisporus* (ATHU–M 5120); **b.** *C. mollis* (ATHU–M 5123); **c.** *C. autochthonus* (ATHU–M 5121). **d.** Yellowing basidiole: *C. mollis* (ATHU–M 5123). **e.** Yellowing, thick-walled cheilocystidia: *C. autochthonus* (ATHU–M 5121). **f.** Disintegrating basidiole and basidium with yellow-brown, strongly granular content: *C. epibryus* (ATHU–M 5106). **g.** Pleurocystidioid body: *C. calolepis* (ATHU–M 5105). **h-j.** Septate, branched and thick-walled cheilocystidia: *C. applanatus* var. *subglobiger* (ATHU–M 5332). **k.** Secretory hyphae in the lamellar trama: *C. mollis* (ATHU–M 5123). Scale bars = 10 μm.

Literature Cited

Aime MC. 1999. Generic concepts in the *Crepidotaceae* as inferred from nuclear large subunit, ribosomal DNA sequences, morphology, and basidiospore dormancy patterns. M.S. Thesis, Virginia Polytechnic Institute and State University, Blacksburg, VA.

Aime MC. 2001. Biosystematic studies in *Crepidotus* & the *Crepidotaceae* (Basidiomycetes, Agaricales). Ph.D Thesis, Virginia Polytechnic Institute and State University. Blacksburg, Virginia.

Aime MC, Vilgalys R, Miller Jr OK. 2005. The *Crepidotaceae* (Basidiomycota, Agaricales): phylogeny and taxonomy of the genera and revision of the family based on molecular evidence. Amer. J. Bot. 92: 74–82.

Avtzis N, Diamandis S. 1988. Insects and fungi of the National Parks of Olympus, Pelion, Prespes and Frakton, Drama. Dasiki Erevna 2: 67–75 (in Greek).

Bandala VM, Montoya L. 2000a. A revision of some *Crepidotus* species related to Mexican taxa. Mycol. Res. 104: 495–506.

Bandala VM, Montoya L. 2000b. A taxonomic revision of some American *Crepidotus*. Mycologia 92: 341–353.

Bandala VM, Montoya L. 2004. *Crepidotus* from Mexico: New records and type studies. Mycotaxon 89 (1): 1–30.

Bandala VM, Montoya L, Moreno G. 1999. Two *Crepidotus* species from Mexico with notes on selected type collections. Mycotaxon 72: 403–416.

Bas C. 1988. Orders and families in agarics and boleti. In: Flora Agaricina Neerlandica, Vol. 1. 40-49. Bas C. et al. eds., AA. Balkema, Rotterdam, Netherlands.

Breitenbach J, Kränzlin F. 2000. Fungi of Switzerland. Vol. 5. Agarics 3rd part. *Cortinariaceae*. Mycological Society of Lucern.

Clémençon H. 1997. Anatomie der Hymenomyceten. F. Flück-Wirth, Teufen.

Diamandis S. 1992. The mushrooms of Greece. Ion, Athens (in Greek).

Diamandis S, Perlerou C. 1990. New recordings of higher Basidiomycetes and Ascomycetes in Greece. Scientific Annals of the Department of Forestry and Natural Environment: 293-303 (in Greek).

Diapoulis CA. 1939. Contribution to the study of the fungi of Mt. Pelion. Rep. Phys. Sci. 50–51: 1–7 (in Greek).

Dimou DM, Zervakis GI, Polemis E. 2002a. Mycodiversity studies in selected ecosystems of Greece: I. Macrofungi from the southernmost *Fagus* forest in the Balkans (Oxya Mountain, Central Greece). Mycotaxon 82: 177–205.

Dimou DM, Polemis E, Zervakis GI. 2002b. Macromycetes associated with *Alnus glutinosa* from Greece. Proceedings of the 11th Hellenic Phytopathological Congress, Preveza, Greece. p. 53.

Ersel FY, Solak MH. 2004. Contributions to the Macrofungi of İzmir Province. Turk. J. Bot. 28: 487-490.

Hawksworth DL, Kirk PM, Sutton BC, Pegler DN. 1995. Ainsworth & Bisby's Dictionary of the Fungi. 8th ed. CAB International, Wallingford, UK.

Hesler LR, Smith AH. 1965. North American species of *Crepidotus*. Hafner Publishing Co., New York.

Jülich W. 1981. Higher taxa of Basidiomycetes. Bibliotheca Mycologica 85. J. Cramer, Vaduz.

Kirk PM, Ansell AE. 1992. Authors of fungal names. Index of fungi supplement. C.A.B. U.K.

Kirk PM, Cannon PF, David JC, Stalpers JA. 2001. Dictionary of the Fungi. 9th ed. CABI Publishing, Wallingford. UK.

Konstantinidis G. 2002. Mushrooms. A fictionary microcosm. Capon, Athens (in Greek).

Krisai-Greilhuber I, Senn-Irlet B, Voglmayr H. 2002. Notes on *Crepidotus* from Mexico and the south-eastern USA. Persoonia 17: 515–539.

Kühner R. 1980. Les Hyménomycètes agaricoides (*Agaricales, Tricholomatales, Pluteales, Russulales*). Étude générale et classification. Bull. Soc. Linn. Lyon 49: 1-1027, numero speciale.

Lonati G. 1993. Fungi Mediterranei rariores. *Crepidotus mollis* var *squamulosus* Coutinho. Mic. Veg. Med. 8: 83-84.

Lonati G. 2000. Il genere *Crepidotus* in area mediterranea. Mic. Veg. Med. 15: 161–186.

Maire R, Politis J. 1940. Fungi Hellenici. Catalogue raisonné des champignons connus jusqu' ici en Grèce. Actes de l'Institute Botanique de l' Université d'Athènes 1: 27–179.

Malençon G, Bertault R. 1975. Flore des champignons supérieurs du Maroc. Tome II. Travaux de l' Institut Scientific Chérifien et de la Faculté des Sciences de Rabat. Série botanique et bologie végétale N° 33. Rabat.

Minter DW. 1988. British Mycological Society Greek Foray. London.

Moncalvo J-M, Vilgalys R, Redhead SA, Johnson JE, James TY, Aime MC, Hofstetter V, Verduin SJW, Larsson E, Baroni TJ, Thorn RG, Jakobsson S, Clémençon H, Miller OK. 2002. One hundred and seventeen clades of euagarics. Mol. Phylogenet. Evol. 23: 357–400.

Moser M. 1978. Keys to Agarics and Boleti (*Polyporales, Boletales, Agaricales, Russulales*). Roger Philips, London.

Nordstein S. 1990. The genus *Crepidotus* (Basidiomycotina, Agaricales) in Norway. Synopsis Fungorum 2, Fungiflora, Oslo.

Ortega A, Buendía AG. 1989. Notas sobre el genero *Crepidotus* (Fr.) Staude en España peninsular. Int. J. Myc. Lich. 4 (1–2): 93–105.

Öztürk C, Kaşik G, Doğan HH, Aktaş S. 2003. Macrofungi of Alanya District. Turk. J. Bot. 27: 303-312.

Pantidou ME. 1991. Mushrooms in the forests of Greece. The Goulandris National History Museum, Athens.

Pilát A. 1948. Monographie des espèces européenes du genre *Crepidotus* Fr. Atl. Champ. Eur. 6. Praga.

Polemis E, Zervakis GI, Dimou DM. 2002. New and interesting findings of macrofungi from the islands of Andros and Naxos (Cyclades, Greece). Book of Abstracts, 7th International Mycological Congress, Oslo. p. 169–170.

Senn-Irlet B. 1993. Type studies in *Crepidotus* II. Persoonia 15: 155–167.

Senn-Irlet B. 1995. The genus *Crepidotus* (Fr.) Staude in Europe. Persoonia 16 (1): 1–80.

Senn-Irlet B, De Meijer A. 1998. The genus *Crepidotus* from the State of Paraná, Brazil. Mycotaxon 66: 165–199.

Sesli E, Denchev CM. 2005. Checklists of the myxomycetes and macromycetes in Turkey. Mycologica Balcanica 2: 119-160.

Singer R. 1947. Monograph of the genus *Crepidotus*. Lilloa 13: 59–95.

Singer R. 1973. The genera *Marasmiellus, Crepidotus* and *Simocybe* in the neotropics. Beih. Nova Hedwigia 44: 1–484.

Singer R. 1986. The Agaricales in modern taxonomy. 4th ed. Koeltz Scientific Books, Koeningstein.

Stangl J, Krieglsteiner GJ, Enderle M. 1991. Die Gattung *Crepidotus* (Fr.) Staude 1857 in Deutschland unter besonderer Berücksichtigung Augsburger Funde. Zeit. Mykol. 57: 117–148.

Tkalčec Z, Mešić A. 2003. Preliminary checklist of Agaricales from Croatia V: Families Crepidotaceae, Russulaceae and Strophariaceae. Mycotaxon 88: 279–314.

Watling R, Gregory NM. 1989. British Fungus Flora. 6. *Crepidotaceae, Pleurotaceae* and other pleurotoid agarics. Royal Botanic Garden, Edinburgh.

Zervakis G, Dimou D, Balis C. 1998. A check-list of the Greek Macrofungi including hosts and biogeographic distribution: I. Basidiomycotina. Mycotaxon 66: 273–336.

MYCOTAXON

Volume 94, pp. 43–46 *October–December 2005*

The lichen flora of the Termessos National Park in Southwestern Turkey

Özge Tufan, Hüseyin Sümbül & Ayşen Özdemir Türk

ozgetufan@akdeniz.edu.tr
Akdeniz University, Faculty of Arts and Sciences, Biology Department,
TR-07058 Antalya, Turkey

Abstract – Between March 2002 and 2003, the lichen flora of the Termossos National Park was studied for the first time. In all, 161 taxa (152 species, 4 subspecies, 5 varieties) were determined from 1114 lichen samples, of which 86 were new to Antalya Province and 5 were new to Turkey. The complete checklist can be downloaded as PDF file from www.mycotaxon.com/resources/weblists/html.

Key words – lichens, Güllük Mountain, Antalya

Introduction

The number of studies on the lichen flora of Turkey has increased significantly over the last two decades (e.g., Aslan 2000, Breuss & John 2004, Çiçek & Özdemir Türk 1998, Güvenç et al. 1996, Özdemır Türk & Güner 1998, Öztürk & Güvenç 2003). Although the lichen flora of the Mediterranean phytogeographical region of Turkey has received more attention than the other regions of Turkey (John 1996, 2003; John & Nimis 1998; Nimis & John 1998; John et al. 2000), even this area needs additional research. To determine the lichen flora of the region, floristic studies that focus on small areas with high biodiversity are needed.

Throughout the Mediterranean region of Turkey, there are ruins and cities from the ancient civilizations, but until now no lichen floristic or biodeterioration study has been published from such places. Termessos (Güllük Mountain) National Park, located on the West side of the Taurus Mountains in Antalya province, southwestern Turkey, is such a site. It is famous for its ancient city, Termessos, which is situated on a natural platform at the top of Güllük Mountain, which has been formed by chemical erosion and tectonic movements. It includes a canyon with very steep walls as high as 500-600 m.

Material and Methods

The research is based on 1114 lichen samples, which were collected from 54 localities (Table 1) in Termessos National Park between March 2002 and September 2003. In every locality coordinates and altitude were measured by GPS (Garmin 12X) and all lichen samples were taken together with their substratum. The samples were brought the laboratory and air-dried under room conditions (25 ± 2 °C, RH 60±10).

For identification, macroscopic and microscopic characters were examined with stereo- and light microscopes and by reference to recent literature (e.g. Wirth 1995, Purvis et al. 1992, Clauzade & Roux 1985, Giordani et al. 2002, Jørgensen 1997, Zeybek et al. 1993, Breuss 1990, Moberg 1977). Following identification, the lichens were deposited in Akdeniz University Herbarium (AKDU).

Results

A PDF file containing the list of lichen species (including locality numbers and substrata) encountered in Termessos National Park, table of localities, and map of the study area can be downloaded from **http://www.mycotaxon.com/resources/ weblists.html**. The abbreviations of authors are in accordance with Brummitt & Powell (1992). Lichen taxa new to Turkey are indicated by *, those new to Antalya province by #.

Discussion

This study reports 161 taxa from Termessos National Park, of which *Collema conglomeratum*, *Lecania inundata*, *Leptogium furfuraceum*, *Peltigera monticola* and *Physconia servitii* are new to Turkey and 86 are new to Antalya province. Although the lichen flora of the Mediterranean region of Turkey is reasonably well studied, it is quite remarkable to find still so many new lichen records for the region as well as some new to the country, emphasizing that much more explorative effort should be made on its lichen flora.

In the study area, in addition to common lichen species for the Mediterranean Region, such as *Lecanora bolcana* and *Diploschistes ocellatus,* we determined "manna" lichens, such as *Aspicilia desertorum* and *A. hispida*, which usually grow in steppes, suboceanic species such as *Degelia plumbea* and *Staurolemma omphalarioides*, and oceanic species, such as *Collema furfuraceum* and *C. nigrescens*. Although the study area is relatively small (ca. 6702 ha), the wide variation in its topology, formed by high mountains, valleys and a deep canyon, evidently provides habitats for a rich lichen biodiversity.

Calcareous species are dominant due to the widespread occurrence of calcareous rocks throughout the study area, and because of the frequency of trees with acidic bark, such as *Pinus nigra* and *Quercus coccifera,* most of the epiphytic species are acidophytic.

Of particular note are *Anthracocarpon virescens, Caloplaca adriatica, Hypocenomyce anthracophila, Neocatapyrenium rhizinosum, Pertusaria hymenea, Placidium pilosellum, Solenopsora liparina* and *Staurolemma omphalarioides,* for which there are only one or two records from Turkey (Pisut 1970; Breuss 1998; Nimis & John 1998; John et al. 2000; John 1996, 2003; Breuss & John 2004).

On the ruins of the ancient city of Termessos, not only species with a wide ecological amplitude, such as *Aspicilia calcarea*, *Caloplaca aurantia*, *Lecanora muralis*, *Lobothallia radiosa*, *Placynthium nigrum* and *Xanthoria elegans* were found, but also species mainly found in the Boreal-Mediterranean region and between the south of Central Europe and the Mediterranean Region such as *Aspicilia farinosa*, *Caloplaca chrysodeta*, *C. xantholyta*, *Collema cristatum*, *Diploschistes ocellatus*, *Lepraria nivalis*, *Solenopsora candicans* and

Solenopsora liparina (Wirth 1995). Because the ancient city was built from local stones, the species on the ruins are similar to the lichen flora found elsewhere in the study area.

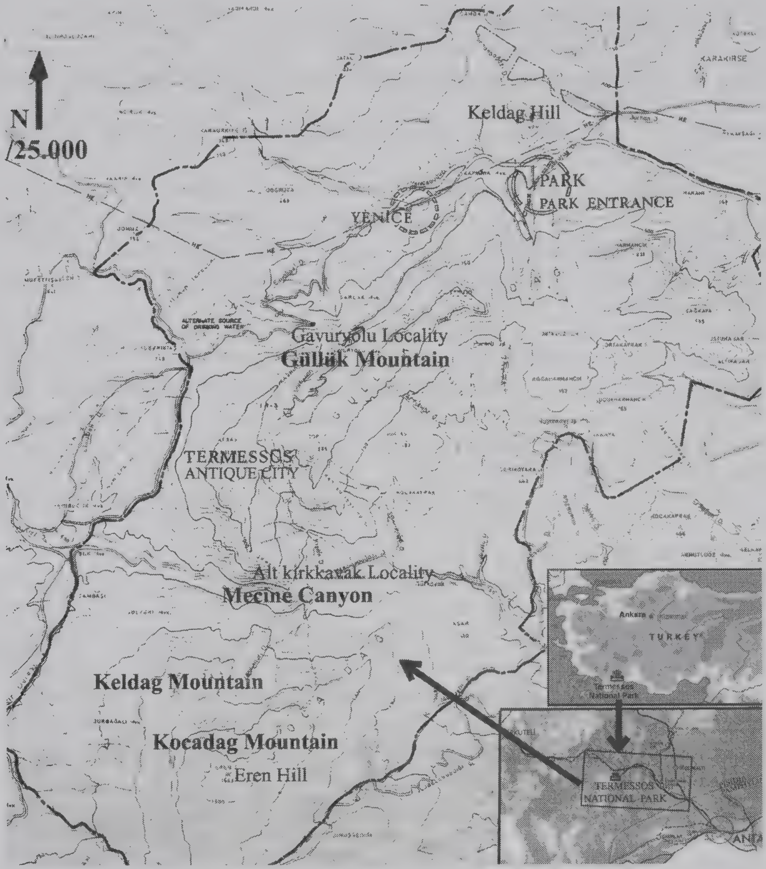

N
/25.000
KARAKIRSE
Keldag Hill
PARK
PARK ENTRANCE
YENICE
ALTERNATE SOURCE OF DRINKING WATER
Gavuryolu Locality
Güllük Mountain
TERMESSOS ANTIQUE CITY
Alt kirkkavak Locality
Mecine Canyon
Keldag Mountain
Kocadag Mountain
Eren Hill
Ankara
TURKEY
Termessos National Park
TERMESSOS NATIONAL PARK

Figure 1: Map of Termessos National Park

Acknowledgements

The authors thank Prof. Mark R. D. Seaward (England) and Dr. Harrie Sipman (Germany) for suggestions and comments that improved the manuscript. The lichen species were collected during the project "The comparison of the lichen floras of the Termessos National Park and Düzlerçamı Region damaged in the July 1997 fire", funded by Akdeniz University Scientific Research Projects Unit (Project number 2002.02.0121.02). We are indebted to Akdeniz University Scientific Research Projects Unit for financial support, to Prof. Dr Per. Magnus Jørgensen (Norway), Prof. Dr Helmut Mayrhofer (Austaria), Prof. Dr Teuvo Ahti (Finland), Assoc. Prof. Dr Roland Moberg (Sweden), Dr Othmar Breuss (Austaria), Dr Orvo Vitikainen (Finland), Dr Brian Coppins (Edinburg), Dr Volker John (Germany) and Alan Orange (Britain) for their identification of lichens, and to the Antalya Directorship of National Parks for granting permission for this study in Termessos National Park.

Literature Cited

Aslan A. 2000. Lichens from the regions of Artvin, Erzurum, and Kars (Turkey). Israel Journal of Plant Sciences 48: 143–155.

Breuss O. 1990. Die Flechtengattung *Catapyrenium* in Europa. Stapfia 23, 110 pp.

Breuss O. 1998. *Catapyrenium* und verwandte Gattungen (lichenisierte Ascomyceten, *Verrucariaceae)* in Asien—eine erste Übersicht. Annalen des Naturhistorischen Museum in Wien, B, 100: 656–669.

Breuss O, John V. 2004. New and interesting records of lichens from Turkey. Österreichische Zeitschrif für Pilzkunde 13: 281–294.

Brummitt RK, Powell CE. 1992. Authors of plant names. Royal Botanical Gardens, Kew, 732 pp.

Clauzade G, Roux C. 1985. Likenoj de Okcidenta Eurupo. Bulletin de la Societé Botanique du Centre-Ouest, Nouvelle série, 893 pp.

Çiçek A, Özdemır Türk A. 1998. Lichen flora of Sakarya province (Turkey). Doğa-Turkish Journal of Botany 22: 99–119.

Giordani P, Nicora P, Rellini I, Brunialti G, Elix JA. 2002,. The lichen genus *Xanthoparmelia* (Ascomycotina, *Parmeliaceae*) in Italy. Lichenologist 34 (3): 189–198.

Güvenç Ş, Aslan A, Öztürk Ş. 1996. The lichen flora of Kapıdağ Peninsula.- In: Öztürk, M. A., Seçmen, Ö. & Görk, G. (eds.) Plant life in southwest and central Asia. Proceedings of the 4 th plant life in southwest Asia symposium held in Izmir 21 - 28 may 1995, Ege Univ. Press, Bornova-Izmir, pp. 472–478.

John V. 1996. Preliminary catalogue of lichenized and lichenicolous fungi of Mediterranean Turkey. Bocconea 6: 173–216.

John V. 2003. Flechten aus der Türkei, von G. Ernst gesammelt. Herzogia 16: 167–171.

John V, Nimis PL. 1998. Lichen flora of Amanos mountain and the province of Hatay. Doğa-Turkish Journal of Botany 22: 257–267.

John V, Seaward MRD, Beatty JW. 2000. A neglected lichen collection from Turkey: Berkhamsted School expedition 1971. Doğa-Turkish Journal of Botany 24: 239–248.

Jørgensen PM. 1997. Further notes on hairy *Leptogium* species. Symbolae Botanicae Upsalienses 32 (1): 113–130.

Moberg R. 1977. The lichen genus *Physcia* and allied genera in Fennoscandia. Symbolae Botanicae Upsalienses 22: 1–108.

Nimis PL, John V. 1998. A contribution to the lichen flora of Mediterranean Turkey. Cryptogamie, Bryologie et Lichénologie 19: 35–58.

Özdemır Türk A, Güner H. 1998, Lichens of the Thrace region of Turkey. Doğa-Turkish Journal of Botany 22: 397–407.

Öztürk Ş, Güvenç Ş. 2003. Lichens from the western part of the Black Sea Region of Turkey. Acta Botanica Hungarica 45: 169–182.

Pisut, I. 1970. Interessante Flechtenfunde aus der Türkei. Preslia (Praha) 42: 379–383.

Purvis OW, Coppins BJ, Hawksworth DL, James PW, Moore DM. 1992. The lichen flora of Great Britain and Ireland. Edmundsbury Press, London, 719 pp.

Wirth V. 1995. Die Flechten Baden–Württembergs. Teil: 1-2, Eugen GmbH & Co., Stuttgart, 1006 pp.Zeybek U., John V, Lumbsch HT. 1993. Türkiye likenlerinden *Hypogymnia* (Nyl.) Nyl. cinsi üzerinde taksonomik araştırma. Doğa-Turkish Journal of Botany 17: 109–116.

Zeybek U, John V, Lumbsch HT. 1993. Türkiye likenlerinden *Hypogymnia* (Nyl.) Nyl. cinsi üzerinde taksonomik araştırma. Doğa-Turkish Journal of Botany 17: 109–116.

MYCOTAXON

Volume 94, pp. 47–50 October–December 2005

Two new species of *Anthracoidea* (*Ustilaginales*) from China

LIN GUO

guol@sun.im.ac.cn
Key Laboratory of Systematic Mycology & Lichenology
Institute of Microbiology, Chinese Academy of Sciences
Beijing 100080, China

Abstract—Two new species, *Anthracoidea setosae* on *Carex setosa* and *A. xizangensis* on *Kobresia duthiei*, are described and compared with *A. misandrae* and related species.

Key words—smut fungi, Ustilaginomycetes, taxonomy

A new species of *Anthracoidea* on *Carex setosa* (Subgen. *Carex*, Sec. *Frigidae*) was discovered from our herbarium (HMAS 67908, 34929, 34930). These specimens were wrongly identified by the author (Guo 1994) as *A. misandrae*. The new species of *Anthracoidea* is similar to *A. misandrae* and *A. sempervirentis* Vánky on host plants in the same section of *Carex* in having ustilospores of the same size. It differs mainly from *A. misandrae* by ustilospores with minute warts measuring 0.125-0.3 μm in diam., while *A. misandrae* has ustilospores with larger warts [(0.2-)0.4-0.8(-1) μm in diam. (Kukkonen 1963: 83)]. It differs mainly from *A. sempervirentis* by ustilospores with regularly distributed warts as seen by SEM (scanning electron microscopy), while *A. sempervirentis* has ustilospores with a "surface by SEM partly with sparse, 0.1-0.2 μm high knobs, partly with abundant and dense, up to 0.5 μm high, irregular, often confluent, rounded warts." (Vánky 1979: 226). The new species is described as:

Anthracoidea setosae L. Guo, sp. nov. Figs. 1-2

Sori in ovariis, subglobosi vel ovoidei, 1.2-2 mm longi, 1-1.8 mm lati, primum membrana cinerascenti, fungali cooperti, deinde expositi. Massa sporarum nigra, semiagglutinata. Ustilosporae a fronte globosae, ellipsoideae, leviter irregulares vel irregulares, 17.5-25(-27) x 12.5-20 μm, ab acie 10-15 μm latae, flavidobrunneae vel atrobrunneae; pariete aequaliter vel inaequaliter incrassato, 1-2.5(-3) μm crasso, tumores interni desunt, regiones lucem repercutientes desunt, superficie minute et dense verruculoso sub SEM.

Sori in ovaries, subglobose or ovoid, 1.2-2 mm long and 1-1.8 mm wide, at first covered by a grayish, fungal membrane, later becoming exposed. Spore mass black, semi-agglutinated. Ustilospores in plane view globose, ellipsoidal, slightly irregular or irregular, 17.5-25(-27) x 12.5-20 μm, in side view 10-15 μm wide, yellowish-brown or blackish-brown; wall evenly or unevenly thickened, 1-2.5(-3) μm, no internal swellings, no light reflective areas, surface minutely and densely verruculose as seen by SEM.

On *Carex setosa* Boott (*Cyperaceae*, Subgen. *Carex*, Sect. *Frigidae*), Gansu: Zhouqu, Shatanlinchang, alt. 3050 m, 4 IX 1992, L. Guo 1276, HMAS 67908 (holotypus hic designatus); Sichuan: Emei Shan, Leidongping, alt. 2500 m, 10 VII 1969, C. M. Wang, Y. X. Han & Q. M. Ma 314, HMAS 34930 (paratypus); Emei Shan, Jindingsi, alt. 3150 m, 9 VII 1969, C. M. Wang, Y. X. Han & Q. M. Ma 296, HMAS 34929 (paratypus).

Etymology: Refers to the host plant *Carex setosa.*

Anthracoidea misandrae was discovered in the Herbarium of the Institute of Botany, Chinese Academy of Sciences (PE) and collected by Prof. Kuan Kechien from Xinjiang Uygur Autonomous Region in 1957. It is described as:

Anthracoidea misandrae Kukkonen, Ann. Bot. Soc. Zool.-Bot. Fenn. „Vanamo" 34(3): 82, 1963. **Figs. 3-4**

Sori in ovaries, ellipsoidal, 2.5-3.5 mm long and 1.5-2.5 mm wide, at first covered by a grayish, fungal membrane, later becoming exposed. Spore mass black, semi-agglutinated. Ustilospores in plane view subglobose, ellipsoidal or ovoid, 17.5-25(-26) x 15-20(-22) μm, in side view 10-14 μm wide, dark reddish-brown; wall evenly thickened, ca. 1 μm, no internal swellings, no light reflective areas, surface verrucose.

On *Carex stenocarpa* Turcz. ex V. Krecz. (*Cyperaceae*, Subgen. *Carex*, Sect. *Frigidae*), Xinjiang: Nilka Xian, 60 Km N of Wulasitai (in the Borohoro Mountains) 31 VIII 1957, K. C. Kuan 3991, HMAS 132710.

Another new species of *Anthracoidea* on *Kobresia duthiei* (Sec. *Elyna*) was discovered from our herbarium (HMAS 67973) and collected by Prof. Zhuang Jianyun in 1990. The specimen was wrongly identified by the author as *A. filifoliae* L. Guo (1995-1996) on the section *Kobresia.* The new species differs from *A. filifoliae* by minute warts on the surface of the ustilospore as seen by SEM and host plants in different sections of the genus *Kobresia*, while *A. filifoliae* has dense and minute warts between the larger warts on the surface of the ustilospores. Only *A. elynae* (Syd.) Kukkonen (1963: 65) has been recorded previously on the section *Elyna*. The new species differs from *A. elynae* by having warts on the surface of the ustilospores, while the surface of the ustilospores of *A. elynae* is smooth. The new species is described as:

Anthracoidea xizangensis L. Guo, **sp. nov.** **Figs. 5-6**

Sori in ovariis, ellipsoidei vel ovoidei, 1-2 mm longi, 0.7-1 mm lati, primum membrana cinerascenti, fungali cooperti, deinde expositi. Massa sporarum nigra, semiagglutinata. Ustilosporae a fronte globosae, ellipsoideae vel ovoideae 17-22.5 x 15-18 μm, ab acie 10-15 μm latae, atrobrunneae; pariete aequaliter incrassato, 1.5-2 μm crasso, tumores interni desunt, regions lucem repercutientes desunt, superficie minute et dense verruculoso sub SEM.

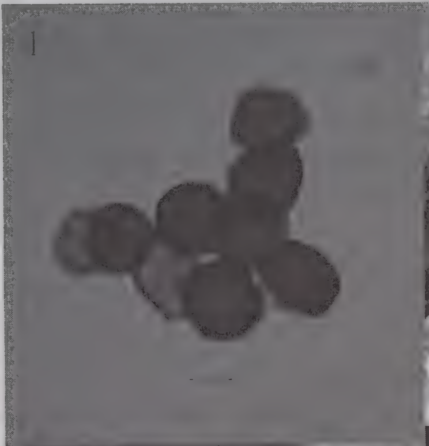
Sori in ovaries, ellipsoidal or ovoid, 1-2 mm long and 0.7-1 mm wide, at first covered by a grayish, fungal membrane, later becoming exposed. Spore mass black, semi-

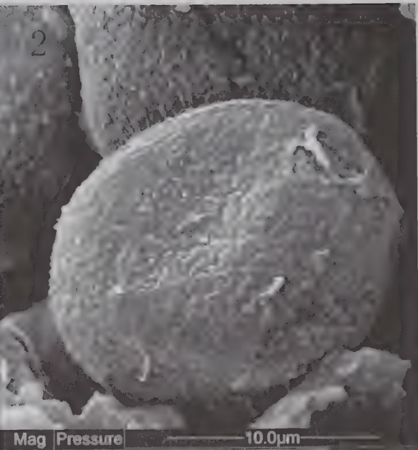
---

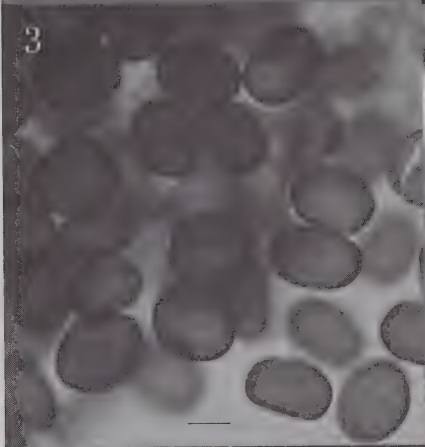
Figs. 1-2. Ustilospores of *Anthracoidea setosae* on *Carex setosa* as seen by LM (light microscopy) and SEM (HMAS 67908, holotypus).

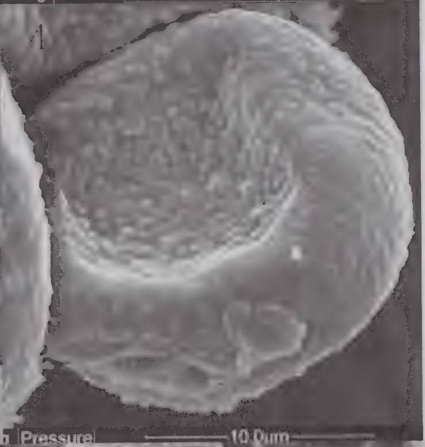
Figs. 3-4. Ustilospores of *Anthracoidea misandrae* on *Carex stenocarpa* as seen by LM and SEM (HMAS 132710).

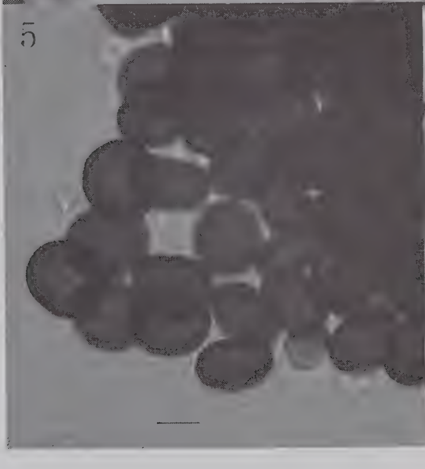
Figs. 5-6. Ustilospores of *Anthracoidea xizangensis* on *Kobresia duthiei* as seen by LM and SEM (HMAS 67973, holotypus). Bars = 10 μm

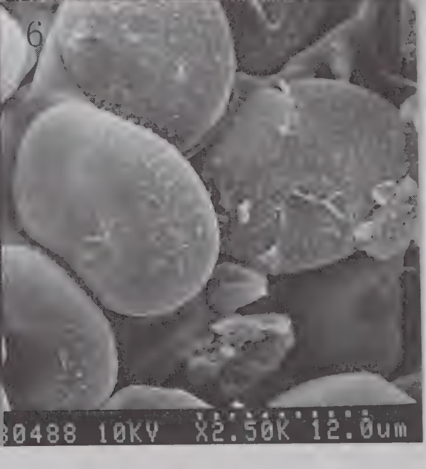

1


2
Mag Pressure
10.0µm


3


4
Pressure
10.0µm


5


6
0488 10KV X2.50K 12.0um

agglutinated. Ustilospores in plane view globose, ellipsoidal, or ovoid, 17-22.5 x 15-18 µm, in side view 10-15 µm wide, blackish-brown; wall evenly thickened, 1.5-2 µm, no internal swellings, no light reflective areas, surface minutely and densely verruculose as seen by SEM.

> On *Kobresia duthiei* C. B. Clarke (*Cyperaceae*, Sect. *Elyna*), Xizang: Dinggye, Yala Shan, alt. 4950 m, 15 VIII 1990, J. Y. Zhuang 2919, HMAS 67973 (holotypus hic designatus).

Etymology: Refers to the locality Xizang Autonomous Region (Tibet).

Since 2000 (Guo 2000), eight new species and three new records of the genus *Anthracoidea* have been recorded in China (Guo 2002, 2004, Guo & Wang 2005, Guo & Zhang 2004, Wang & Piepenbring 2002, Zhang & Guo 2004), including *A. setosae* and *A. xizangensis* (in this paper).

Acknowledgements

The author would like to express her deep thanks to Drs. Vánky and Chlebicki for reading the manuscript and serving as pre-submission reviewers, to Dr. Pennycook for nomenclature review, to Profs. Liang Songjun and Zhang Shuren (Institute of Botany, Chinese Academy of Sciences) for identifying the host plants, and to Mrs. Xie Jiayi for assistance with SEM photographs. This study was supported by the National Natural Science Foundation of China (No. 30230020 and No. 30270016).

Literature Cited

Guo L. 1994. *Anthracoidea* and allied genera in China (*Ustilaginales*). Mycosystema 7: 89-104.

Guo L. 1995-1996. *Anthracoidea filifoliae* sp. nov. and four smut species new to China. Mycosystema 8-9: 163-168.

Guo L. 2000. Flora Fungorum Sinicorum, *Ustilaginaceae* Vol. 12, Science Press, Beijing (China). 124 pp.

Guo L. 2002. Two new species of Ustilaginomycetes and a species new to China. Mycotaxon 82: 147-150.

Guo L. 2004. *Anthracoidea shaanxiensis* sp. nov. (*Ustilaginales*) from China. Nova Hedwigia 79: 507-509.

Guo L, Wang SR. 2005. A new species and a new record of *Anthracoidea* (*Ustilaginales*) from China. Mycotaxon 93: 159-162.

Guo L, Zhang HC. 2004. A new species and two new records of Ustilaginomycetes from China. Mycotaxon 90: 387-390.

Kukkonen I. 1963. Taxonomic studies on the genus *Anthracoidea (Ustilaginales).* Ann. Bot. Soc. Zool.-Bot. Fenn. “Vanamo” 34(3): 1-122.

Vánky K. 1979. Species concept in *Anthracoidea* (*Ustilaginales*) and some new species. Bot. Not. 132: 221-231.

Wang SR, Piepenbring M. 2002. New species and new records of smut fungi from China. Mycol. Progr. 1: 399-408.

Zhang HC, Guo L. 2004. Two new species and a new record of *Anthracoidea* (*Ustilaginales*) from China. Mycotaxon 89: 307-310.

MYCOTAXON

Volume 94, pp. 51–54 October–December 2005

A new species of *Lepiota* (*Agaricaceae*, Basidiomycetes) from China

HAN-CHEN WANG

higherfungi2005@yahoo.com.cn
Kunming Institute of Botany, Chinese Academy of Sciences
Kunming 650204, Yunnan Province, P. R. China
Institute of Applied Ecology, Chinese Academy of Sciences
Shenyang 110016, Liaoning Province, P. R. China
Graduate School of the Chinese Academy of Sciences, P. R. China

ZHU-LIANG YANG*

fungi@mail.kib.ac.cn
Kunming Institute of Botany, Chinese Academy of Sciences
Kunming 650204, Yunnan Province, P. R. China

Abstract—A new species, *Lepiota catenariocystidiata*, is described and illustrated. It is compared with similar species.

Key words—*Agaricales*, taxonomy

Introduction

During our study of lepiotaceous fungi, we came across an undescribed species of the genus *Lepiota* (Pers.: Fr.) Gray. It is described and illustrated herein. In descriptions of the basidiomata, color designations (e.g., 1A1) are from Kornerup & Wanscher (1981), and color names with first letters capitalized (e.g., Pale Smoke Gray) are from Ridgway (1912). In descriptions of basidiospores, the notation [*n*/*m*/*p*] shall mean *n* basidiospores measured from *m* basidiomata of *p* collections in Melzer's reagent. *Q* is used to mean quotient of length and width of a spore in side view; **Q** means average *Q* of all basidiospores ± sample standard deviation. Herbarium code HKAS = Herbarium of Cryptogams, Kunming Institute of Botany, Chinese Academy of Sciences.

Taxonomy

Lepiota catenariocystidiata Han C. Wang & Zhu L. Yang, **sp. nov.** Figs. 1-4

Pileus 3-5 cm latus, initio subcampanulatus, deinde convexus vel applanatus, albidus vel griseolus, squamulis tomentosis, griseis vel obscure griseis. Lamellae liberae, albidae vel cremeae. Stipes 4-6 × 0.3-0.6 cm, subcylindricus, albidus, annulatus, squamulis confertis,

---

* *Corresponding author*

tomentosis, griseis infra annulum. Annulus superus, membranaceus. Basidiosporae (7.0) 7.5-9.0 (10.0) × (3.5) 4.0-4.5 (5.0) µm, oblongae vel subcylindricae, incoloratae, hyalinae, dextrinoideae. Basidia 20-30 × 7.5-10 µm, subclavata, 4-sporigera, raro 2-sporigera. Pleurocystidia nulla. Cheilocystidia 10-20 × 8-12 µm, subglobosa, ovata vel breviclavata, catenaria, incolorata. Squamulae pilei ex hyphis subcylindricis terminalibus compositae. Fibulae praesentes.

Holotype: CHINA, Yunnan Prov., Mengla County, Menglun, 2. XI. 1989, Z.L. Yang 918 (HKAS 22145).

Etymology: Named because of the cheilocystidia often in chains.

Basidiomata (Fig. 1) scattered. *Pileus* 3-5 cm in diam, at first subcampanulate, then convex to applanate, with an obtuse umbo or non-umbonate; pileal surface dry, whitish to grayish (1A1-1B1; Pale Smoke Gray) but with pinkish tinge (11A2-11B2; Light Brownish Drab) at center, densely covered with minute, dark gray (11C1 + 11D1 + 11E1; Dark Neutral Gray to Blackish Slate) tomentose squamules over disc, with small, concentrically arranged, grey to dark grey squamules towards the margin, margin often slightly exceeding lamellae. *Lamellae* free, whitish to cream-coloured, moderately crowded, in 2-3 ranks, up to 0.7 cm broad, with white to concolorous eroded edge. *Stipe* 4-6 × 0.3-0.6 cm, central, subcylindrical, hollow, slightly enlarged near base, surface whitish, lower part covered with grey (11C1 + 11D1; Dark Neutral Gray to Hair Brown) tomentose squamules often in belts. *Annulus* superior, membranous, upper surface white and glabrous, lower surface covered with grey tomentose squamules, persistent or fugacious. *Context* whitish, unchanging; odor indistinct; taste slightly hot.

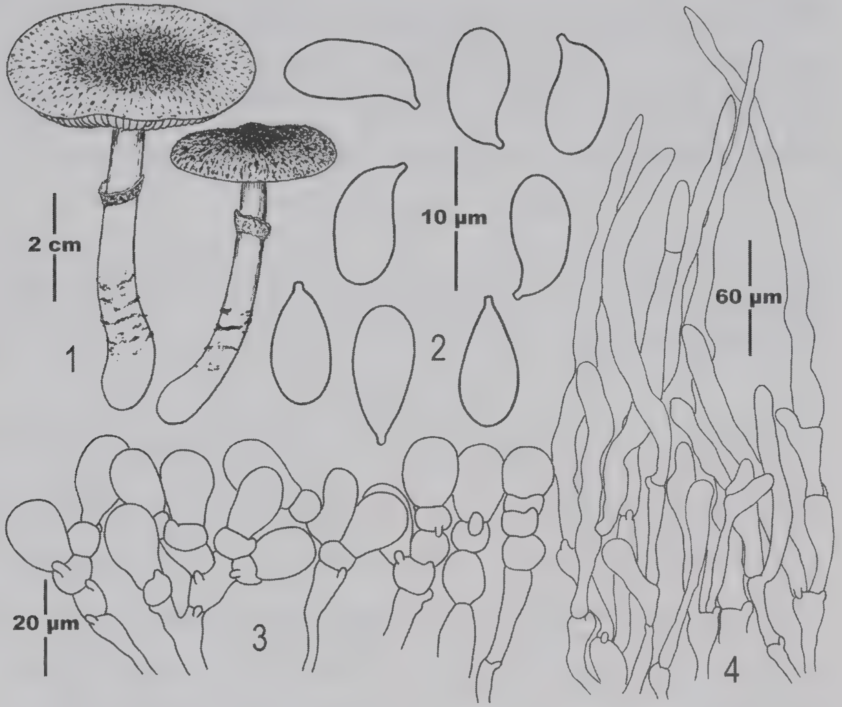
Basidiospores (Fig. 2) [42/2/2] (7.0) 7.5-9.0 (10.0) × (3.5) 4.0-4.5 (5.0) µm [Q = (1.67) 1.75-2.25, **Q** = 2.01 ± 0.18], oblong to subcylindrical, with a distinct suprahilar depression in side view, water-drop-shaped in front view, broader at apical part, attenuate towards the base, slightly thick-walled, colorless, hyaline, dextrinoid, reddish in Congo Red, not metachromatic in Cresyl Blue. *Basidia* 20-30 × 7.5-10 µm, subclavate, hyaline, thin-walled, 4-spored, rarely 2-spored. *Pleurocystidia* absent. *Cheilocystidia* (Fig. 3) abundant, 10-20 × 8-12 µm, subglobose, ovoid to short clavate, often in chains, colorless and hyaline. *Squamules* (Fig. 4) on pileus a disrupted trichodermium consisting of loose fascicles of long, more or less erect, subcylindrical, terminal elements (45-300 × 10-17 µm) with tapering or round apex and pale yellow-brown to dark yellow-brown intracellular pigments; with short clavate cells at the base of these long elements; the repent hyphae at the base of erect elements sometimes with incrusting pigments. Squamules on surface of stipe similar to those on pileus. *Clamp connections* abundant in basidiomata.

Habitat: On well-rotten wood with soil in limestone monsoon forests; fruiting in summer and autumn in southwestern China at 600–700 m elev.

Known distribution: Known from tropical Yunnan only.

Additional material examined: CHINA, Yunnan Prov., Mengla County, Menglun Nature Reserve, 22. X. 1988, Zang 11515 (HKAS 20358).

Notes: *Lepiota catenariocystidiata* is well characterized by its whitish to grayish pileus with a pinkish center, gray to dark gray squamules on the pileus made up of a disrupted trichodermium with long subcylindrical terminal elements and short clavate cells at the base of these long elements, water-drop-shaped basidiospores in front view, catenulate cheilocystidia and the common presence of clamp connections in the basidiomata.



Figs. 1-4. *Lepiota catenariocystidiata* (from holotype)
1. Basidiomata. **2.** Basidiospores. **3.** Cheilocystidia. **4.** Squamules on pileus

Due to the trichodermium type of the squamules on the pileus and the oblong to subcylindrical basidiospores, *L. catenariocystidiata* may belong to *Lepiota* sect. *Ovisporae* (J.E. Lange) Kühner. Species with pileal squamules made up of long, erect elements and short, clavate elements in between were put in the subsect. *Felininae* Bon within sect. *Ovisporae* (Vellinga 2001). Because *L. catenariocystidiata* has short, clavate elements at the base of long, erect ones, it should be placed in the subsection. However, according to recent molecular phylogenetic studies, the ITS data set does not support sect. *Ovisporae*, and thus a re-evaluation of this section is needed (Vellinga 2003). The present species may be clustered within the Clade 1 of *Lepiota* s. l. (Vellinga 2003).

Lepiota catenariocystidiata may be related to *L. felina* (Pers.) P. Karst., *L. pseudolilacea* Huijsman and *L. pseudohelveola* Kühner ex Hora. However, the latter two species have differently coloured basidiomata, ellipsoid to oblong basidiospores in front view and rarely with a suprahilar depression in side view, and differently shaped cheilocystidia (Kühner 1936; Huijsman 1947; Hora 1960; Enderle & Krieglsteiner 1989; Bon 1981, 1996; Candusso & Lanzoni 1990). According to Vellinga (2001), *L. pseudohelveola* should be regarded as a synonym of *L. pseudolilacea*. *Lepiota felina* differs from *L. catenariocystidiata* in the colour of the basidiomata, the shape of the cheilocystidia and basidiospores (Kühner 1936; Huijsman 1947; Hora 1960; Bon 1981, 1996; Enderle & Krieglsteiner 1989; Candusso & Lanzoni 1990; Vellinga 2001).

The basidiospores of *L. catenariocystidiata* are very similar to those of *L. plumbicolor* (Berk. & Broome) Sacc., originally described from Sri Lanka. However, the latter has blackish purple squamules on pileus with elongate clavate terminal elements with an obtusely rounded apex and clavate-cylindrical cheilocystidia (Pegler 1972, 1986).

Acknowledgements

We are very grateful to Dr. E.C. Vellinga for having sent us her valuable publications on *Agaricaceae*, and to Drs. E.C. Vellinga and D.E. Desjardin for their critical reviewing the manuscript. This project was financed by the Natural Science Foundation of China's Yunnan Province (No. 2002C0059M) and the National Natural Science Foundation of China (No. 30270017).

Literature cited

Bon M. 1981. Clé monographique des "Lépiotes" d'Europe (= *Agaricaceae*, Tribus *Lepioteae* et *Leucocoprineae*). Doc. Mycol. 11 (43): p1-p77.

Bon M. 1996. Die Großpilzflora von Europa 3. *Lepiotaceae* (übersetzt und bearbeitet von F. Medjebeur-Thrun & W. U. Thrun). IHW-Verlag: Eching (Germany). 141pp.

Candusso M, Lanzoni G. 1990. *Lepiota* s. l. Fungi Europaei 4. Giovanna Biella: Saronno (Italy). 743pp.

Enderle M, Krieglsteiner GJ. 1989. Die Gattung *Lepiota* (Pers.) S. F. Gray emend. Pat. in der Bundesrepublik Deutschland (Mitteleuropa). Z. Mykol. 55: p43-p104.

Hora FB. 1960. New check list of British Agarics and Boleti. Transactions British Mycol. Soc. 43: p440-p459.

Huijsman HSC. (1947). *Lepiota pseudolilacea* nov. spec. Bull. Soc. Linn. Lyon 16: p180-p183.

Kornerup A, Wanscher JH. 1981. Taschenlexikon der Farben. 3. Aufl. Muster-Schmidt Verlag: Zürich (Switzerland). 242pp.

Kühner MR. 1936. Recherches sur le genre *Lepiota*. Bull. Soc. Mycol. France 52: p175-p238.

Pegler DN. 1972. A revision of the genus *Lepiota* from Ceylon. Kew Bull. 27: p155-p202.

Pegler DN. 1986. Agaric Flora of Sri Lanka. Kew Bull. Add. Ser. 12: p1-p519.

Ridgway R. 1912. Color Standards and Color Nomenclature. R. Ridgway, Washington, D.C. (USA). 43pp.

Vellinga EC. 2001. *Lepiota* (Pers. : Fr.) S. F. Gray. In: Flora Agaricina Neerlandica 5 (eds. M.E. Noordeloos, Th.W. Kuyper and E.C. Vellinga). A. A. Balkema Publishers, Holland: p109-p151.

Vellinga EC. 2003. Phylogeny of *Lepiota* (*Agaricaeae*) - Evidence from nrITS and nrLSU sequences. Mycol. Progr. 2: p305-p322.

MYCOTAXON

Volume 94, pp. 55–73 *October–December 2005*

ITS sequence analysis and ascomatal development of *Pseudogymnoascus roseus*

Y. Jiang & Y. -J. Yao*

yaoyj@sun.im.ac.cn
Systematic Mycology and Lichenology Laboratory, Institute of Microbiology
Chinese Academy of Sciences, Beijing 100080, China

Abstract—ITS sequence analysis and ascomatal development of *Pseudogymnoascus roseus* strains isolated from sclerotia of *Cordyceps sinensis* collected from the Tibetan Plateau, China, are reported in this paper. The ITS sequences of three strains from different locations were identical and were compared with sequences obtained from the BLAST search in GenBank. The strains display the same morphology as the reference collection deposited in K, matching the species description of *P. roseus*. Ascomatal development of the *P. roseus* strains is described. Ascomata of *P. roseus* were found to comprise an aggregation of asci from several different ascomatal initials enveloped by a loose, thick-walled hyphal network. In the parsimony analysis, ITS sequences of *P. roseus* and other *Myxotrichaceae* grouped outside the *Onygenales* and clustered with those of discoid fungi. Members of *Myxotrichaceae* were considered closely related to discomycetes, but greatly diverged from onygenalean fungi. *Myxotrichaceae* did not form a monophyletic group in the ITS tree.

Key words—DNA, fungal culture, taxonomy

Introduction

Pseudogymnoascus Raillo, a genus of Ascomycetes established with two species in 1929, has been referred to either *Gymnoascaceae* Baran. (e.g. Kuehn 1958, Arx 1971, Alexopoulos & Mims 1979, Orr 1979, Benny & Kimbrough 1980, Eriksson & Hawksworth 1986, 1993) or to *Onygenaceae* Berk. (Arx 1987). However, it was placed by Currah (1985) in *Myxotrichaceae* Locq. ex Currah, based on cellulose degradation capacity, smooth ascospores and rhexolytically dehiscing conidia. The latter taxonomic treatment has been widely accepted (Alexopoulos et al. 1996, Kirk et al. 2001). Species of this genus have yellow or rose, globose to subglobose, discrete or confluent ascomata; ascomatal peridium composed of a network of slightly thick-walled hyphae; appendages simple and not distinct; asci globose to ellipsoid, normally 8-spored; ascospores ellipsoid to fusoid, smooth, hyaline, yellow, orange to pink (Cejp & Milko 1966, Orr 1979, Currah 1985).

In addition to the original two species of *Pseudogymnoascus*, *P. roseus* and *P. vinaceus* Raillo, several more species have since been described, e.g. *P. caucasicus* Cejp & Milko,

---

* Author for correspondence

P. bhattii Samson, *P. alpinus* E. Müll. & Arx and *P. dendroideus* Locq.-Lin. However, Samson (1972) considered *P. roseus* and *P. vinaceus* identical and lectotypified the genus with *P. roseus*. Orr (1979) discussed the status of the genus and recognised two species, *P. vinaceus* and *P. roseus*, listing *P. bhattii* as a synonym of *P. vinaceus*. In his monographic study of *Onygenales*, Currah (1985) treated both *P. vinaceus* and *P. bhattii* as synonyms of *P. roseus*. Although Samson's (1972) choice of *P. roseus* as the type species has been followed by others (Arx 1974, 1981, 1987, Currah 1985, Sigler et al. 2000), the genus was also typified by *P. vinaceus* early (Kuehn 1958, Orr 1979).

As more species of *Pseudogymnoascus* were described, the range of morphological characters became more diverse. For example, *P. alpinus* was described as having navicular-fusoid ascospores, and ascomata with branched and recurved appendages (Müller & Arx 1982); *P. dendroideus* with ramified ascomatal appendages and striated ascospores (Locquin-Linard 1982); and *P. roseus* var. *ornatus* Udagawa & Uchiy. with irregularly lobate-reticulate ascospores (Udagawa & Uchiyama 1999). Further, a weakly cellulolytic species having fusoid ascospores with a longitudinal sigmoid crest was assigned to the genus as *Pseudogymnoascus* sp. (Lumley et al. 2000).

Anamorphs of *Pseudogymnoascus* have been referred to *Geomyces* Traaen (Orr 1979, Currah 1985). *Geomyces pannorum* var. *vinaceus* (Dal Vesco) Oorschot is considered as the anamorph of *P. roseus* (Sigler & Carmichael 1976, Oorschot 1980).

Systematically, *Myxotrichaceae* was placed in the order *Onygenales*, along with *Arthrodermataceae* Locq. ex Currah, *Gymnoascaceae* and *Onygenaceae*, by Currah (1985). Species of *Myxotrichaceae* are saprobic, cellulose-degrading, and usually inhabit forest soils and decay plant material. Members of *Gymnoascaceae* do not exhibit strong substrate preferences, and are neither keratinolytic nor cellulolytic. The *Arthrodermataceae* and the *Onygenaceae* degrade keratin and usually inhabit soils enriched with keratin or dung. Currah (1994) further restricted the *Onygenales* to keratinolytic genera and suggested that the *Myxotrichaceae* might represent a distinct evolutionary line from typical members of *Onygenales* and derive from the inoperculate discomycetes and that it merited a placement in its own order. The distant relationship between the *Myxotrichaceae* and other members of *Onygenales* has been confirmed by recent studies (Sugiyama et al. 1999, Mori et al. 2000, Kirk et al. 2001).

Recently, molecular approaches have been introduced to the systematic study of *Myxotrichaceae* and related fungi (Bowman & Taylor 1993, LeClerc et al. 1994, Hambleton et al. 1998, Sugiyama et al. 1999, Sugiyama & Mikawa 2001). Some molecular evidence indicates that *Myxotrichaceae* are distantly related to other members of *Onygenales* (Sugiyama et al. 1999, Mori et al. 2000). Sugiyama et al. (1999) examined the molecular systemtatics of onygenalean taxa (including *P. roseus* and members of *Amauroascaceae* Arx, *Arthrodermataceae*, *Gymnoascaceae* and *Onygenaceae*) based on 18S rDNA sequences and suggested that the *Myxotrichaceae* should be placed in an independent position among the *Helotiales* and the *Erysiphales*, on a different lineage from the keratin-degrading fungi. Based on sequences of 18S rDNA and partial sequences of 28S rDNA, Mori et al. (2000) demonstrated that the *Myxotrichaceae* was distantly related to the other onygenalean families, as a sister group to the *Erysiphales* and that the *Erysiphales*/*Myxotrichaceae* clade was also closely related to some discomycetous fungi, e.g. *Helotiales* and *Thelebolaceae* (Brumm.) Eckblad. *Pseudogymnoascus* has also been used as the outgroup in other molecular systematic studies, e.g. Hambleton et al. (1998)

and Sugiyama & Mikawa (2001). The molecular research has provided more evidence to support the idea that the *Myxotrichaceae* is not closely related to onygenalean fungi.

Ascomatal development of *Pseudogymnoascus* has been described in some taxonomic studies (Samson 1972, Locquin-Linard 1982, Müller & Arx 1982, Tsuneda 1982, Ito & Yokoyama 1987). Samson (1972) described the ascomatal initials as borne on the vegetative hyphae, consisting of coiled ascogonia and producing loose wefts of ascogenous hyphae inside hyphal tufts, and illustrated the ascomatal initials and the ascus formation. When describing the new species *P. alpinus*, Müller & Arx (1982) indicated that ascomatal initials grew as aerial branches of vegetative hyphae, loosely interwoven and sympodially branched once or twice. Locquin-Linard (1982) provided three drawings to describe the different stages of ascomatal develolpment of *P. dendroideus*. Tsuneda (1982) illustrated the development of ascomata in a scanning electron microscopic study of *P. roseus* and Ito & Yokoyama (1987) provided a photograph of the ascomatal initial but without description. The process of ascomatal development in *Pseudogymnoascus* has not yet been described in detail.

Pseudogymnoascus roseus is a species of worldwide distribution frequently found in soil, usually from alpine or forest areas (Christensen et al. 1962, Orr 1979, Ito & Yokoyama 1985, 1987, Currah 1985, Yokoyama et al. 1989, Udagawa & Uchiyama 1999), and occasionally on dung (Ellis & Ellis 1988). It has been also reported from tropical regions (Farrow 1954, Siddiqi 1964, as *P. vinaceus*). *Pseudogymnoascus roseus* was also reported to form mycorrhizal associations with *Vaccinium angustifolium* Aiton in the laboratory (Dalpé 1989), and typical ericoid mycorrhiza with salal (*Gaultheria shallon* Pursh), and also to degrade cellulose and to use organic forms of nitrogen (Xiao & Berch 1995).

During fieldwork undertaken on the Tibetan Plateau for investigation of the Chinese Caterpillar Fungus (*Cordyceps sinensis*, a well-known fungus used as a tonic in traditional Chinese medicine), some other fungi were also isolated from sclerotia of the fungus. Among them, three strains displayed different characters from those of *C. sinensis* in culture. Sequences of internal transcribed spacers (ITS) in the nuclear ribosomal DNA (nrDNA) were obtained for molecular systematic analysis and observations on the cultures were made to elucidate the development of ascomata in these strains. The taxonomic position of the strains was determined as *P. roseus* based on both molecular data and morphological observation. The results are reported here to provide further information on the biology of this fungus.

Materials and Methods

Fungal cultures

Fungal cultures used in this study were isolated from *C. sinensis* specimens, collected from Xiaojin County, Sichuan Province, and Yulong Snow Mountain, Yunnan Province of China, on the southeast of the Tibetan Plateau. Soil and plant debris on the fresh specimens were removed and the surface of the specimens was sterilised with 70% ethanol before isolation. The exoskeleton of the host larva was peeled off by using a scalpel and small pieces of the inner tissue of sclerotium of *C. sinensis* were transferred to fresh slopes of bran agar-peptone medium (potato dextrose agar (PDA) supplemented with 5 % wheat bran and 0.5 % peptone). A few pieces of the exoskeleton were also used as inocula for comparison. Pure cultures were obtained by sub-culturing hyphal tips of primary isolates. Living cultures were maintained at the Institute of Microbiology, Chinese Academy of Sciences, and dried voucher specimens are deposited in HAMS (Chinese Academy of Sciences, Beijing, China) and K (Royal Botanic Gardens, Kew, UK). The details of strains used in this study are listed in Table 1. The cultures were kept at 4 °C for 3–16 weeks for morphological observation and molecular experiments.

DNA extraction

Samples of fresh mycelium were obtained by scraping the culture from the surface of the nutrient slopes after 6 weeks of incubation. DNA extraction was carried out following a modification of Yao et al. (1999). About 0.1 g of fresh mycelium (including some agar) was ground into powder in liquid nitrogen and transferred to a 1.5 ml tube. The lysis buffer of 600 µl 2% CTAB was added, followed by incubation in a water bath at 65 °C for 1h or more. An equal volume of phenol/chloroform/isoamylol (25:24:1) was added and mixed, then centrifuged at 13000 rpm for 10 min. The supernatant was transferred to a fresh 1.5 ml tube, followed by extraction of the chloroform/isoamylol alcohol (24:1). After centrifugation, the supernatant was transferred to a fresh tube and 250 µl isopropanol was added to precipitate the DNA at –20 °C for 4 h or overnight. The precipitate was centrifuged at 13000 rpm for 10 min, then the liquid was drained off and the tube dried at room temperature for more than 2 h. The DNA preparation was resuspended in 40 µl of sterile deionised water. The crude extracts containing unquantified DNA amounts were used as templates for PCR amplification. Dilution of these extracts 2–10 times was sometimes required for successful DNA amplification.

PCR amplification and sequencing

The entire ITS region of nrDNA, including ITS1, ITS2 and 5.8S gene, was amplified by polymerase chain reaction (PCR) utilizing the ITS5/4 primers (White et al. 1990). The amplification was performed in 25 µl volumes of reaction mixture containing: 10 mM Tris/HCl (pH 8.3), 2.5mM $MgCl_2$, 0.2 mM of each of the four deoxyribonucleotide triphosphates, 0.4 µM of each of the two primers, 24 U ml^{-1} Taq polymerase (Sino-American Biotechnology Co.), 1 µl of DNA template (some of them were diluted from the crude DNA extracts). The PCR was performed with an initial denaturation of 97 °C for 2.5 min and 35 cycles of 97 °C for 30 sec, 50 °C for 1 min, 72 °C for 1.5 min and final 72 °C for 10 min.

Products were purified using Watson's PCR Purification Kit (Watson Ltd). Sequencing was performed by the cyclic reaction termination method using fluorescently labelled dideoxyribonucleotide triphosphates, according to the manufacturer's protocols on the Geneamp PCR System 2400 or 9700 (Perkin-Elmer). The sequencing products were purified by ethanol precipitation according to the sequencing kit protocol (ABI Prism® BigDye™ Terminator Cycle Sequencing Ready Reaction Kit, Original and Version 2.0, ABI). Sequencing was performed on an ABI Prism® 3100 Genetic Analyzer (Applera Corporation) and data collected on a Dell computer with the DNA Sequencing Analysis programme (ABI Prism®DNA Sequencing Analysis Software™, Version 3.7). Each fragment was sequenced in both directions for confirmation and the two strands of sequences were assembled with Seqscape programme (ABI Prism® SeqScape Software™, Version 1.1).

Table 1. Test strains of *Pseudogymnoascus roseus* and *Cordyceps sinensis* used in this study.

Isol. #	Fungus	Location	Elev.	Isolation source	Coll. date	Voucher	GenBank Access. #
CS 6-61	*Pseudogymnoascus roseus* Raillo	Xiaojin County, Sichuan	3700m	Sclerotium of *Cordyceps sinensis*	8 June, 2000	HMAS 79435	AY608923
CS 20	*Pseudogymnoascus roseus*	Yulong Snow Mountain, Yunnan	4060m	Exoskeleton of *C. sinensis* host larva	31 May, 2001	HMAS 79436	AY608924
CS 22	*Pseudogymnoascus roseus*	Yulong Snow Mountain, Yunnan	4060m	Sclerotium of *C. sinensis*	31 May, 2001	HMAS 79438 K(M) 108601	AY608922
CS 18	*Cordyceps sinensis* (Berk.) Sacc.	Yulong Snow Mountain, Yunnan	4060m	Sclerotium of *C. sinensis*	31 May, 2001	HMAS 79439	AY608925

DNA sequence analysis and molecular identification of the strains

ITS sequences obtained from this study were compared with existing sequences in GenBank by BLAST database search (Altschul et al. 1997). Additional ITS sequences from *Myxotrichaceae* (*Myxotrichum arcticum*) and from families in the *Onygenales* (including *Gymnoascus* Baran. of *Gymnoascaceae, Ajellomyces* McDonough & A. L. Lewis of *Onygenaceae, Amauroascus* J. Schröt. of *Onygenaceae* and *Arthroderma* Curr. of *Arthrodermataceae*), two representatives of pyrenomycetes, *Neurospora crassa* Shear & B. O. Dodge and *Erysiphe cichoracearum*, and two species of basidiomycetes were also retrieved from GenBank. One of the *C. sinensis* strains among the isolates obtained from this study was also included for ITS sequence analysis. All the sequences from GenBank are listed in Table 2. Sequences were initially aligned with BioEdit 5.0.6 (Hall 1999) and analysed in PAUP 4.0b10 for Macintosh (Swofford 2001). Extra bases of the ITS fragment in several sequences from Genbank were edited. The alignment was further manually adjusted to reduce some obvious mismatch of sequences created by computer alignment.

A total of 29 sequences was included in the analysis and a data matrix containing 761 base pairs of nucleotides was established. A few dozen bases at both ends were excluded from the analysis owing to uncertainty in determining the sequence. Heuristic searches (Swofford & Olsen 1990, Maddison 1991), including TBR (tree bisection-reconnection) swapping for 1000 replicates of random taxon addition using equal weights were used to explore the set of possible trees from many starting points. Ten trees were saved at each replicate. Nucleotide substitutions were treated as unordered and alignment gaps as missing. Relative supports were assessed by bootstrapping (Felsenstein 1985) using equally weighted characters for 1000 replicates. The tree was rooted with the two basidiomycetes, *Agaricus bisporus* and *Ustilago maydis*.

Table 2. ITS sequences from GenBank used for sequence analysis.

Fungus	Taxonomic position	Accession #
Agaricus bisporus (J. E. Lange) Imbach	*Agaricaceae; Agaricales*	AF465404
Ajellomyces capsulatus (Kwon-Chung) McGinnis & Katz	*Onygenaceae; Onygenales*	AF038353
Amauroascus mutatus (Quél.) Rammeloo	*Onygenaceae; Onygenales*	AJ271565
Arthroderma persicolor (Stockdale) Weitzman et al.	*Arthrodermataceae; Onygenales*	AJ000614
Bisporella citrina (Batsch) Korf & S.E. Carp.	*Helotiaceae; Helotiales*	AF335454
Dermea viburni J. W. Groves	*Dermateaceae; Helotiales*	AF141163
Elytroderma deformans (Weir) Darker	*Rhytismataceae; Rhytismatales*	AF203469
Erysiphe cichoracearum DC.	*Erysiphaceae; Erysiphales*	AF011295
Gelatinipulvinella astraeicola Hosoya & Y. Otani	*Helotiaceae; Helotiales*	U72611
Geomyces asperulatus Sigler & J. W. Carmich.	Anamorph	AJ390390
Geomyces pannorum (Link) Sigler & J. W. Carmich.	Anamorph	AJ509872
Geomyces pannorum	Anamorph	AF015789
Geomyces pannorum (Link) Sigler & J. W. Carmich. var. *pannorum*	Anamorph	AF307760
Gymnostellatospora japonica Udagawa et al.	*Myxotrichaceae*	AF062818
Gymnoascus petalosporus (G. F. Orr et al.) Arx	*Gymnoascaceae; Onygenales*	AJ315829
Gymnoascus punctatus (B. G. Dutta & G. R. Ghosh) Arx	*Gymnoascaceae; Onygenales*	AJ315825
Myxotrichum arcticum Udagawa et al.	*Myxotrichaceae*	AF062810
Neofabraea malicorticis H. S. Jacks.	*Dermateaceae; Helotiales*	AF141189
Neurospora tetrasperma Shear & B. O. Dodge	*Sordariaceae; Sordariales*	AF388929
Pezicula ocellata (Pers.) Seaver	*Dermateaceae; Helotiales*	AF141199
Pseudogymnoascus roseus	*Myxotrichaceae*	AF062819
Pseudogymnoascus roseus	*Myxotrichaceae*	AF081431
Scleromitrula spiraeicola (Dennis) T. Schumach. & Holst-Jensen	*Rutstoemiaceae; Helotiales*	Z81448
Sclerotinia trifoliorum Erikss.	*Sclerotiniaceae; Helotiales*	Z99676
Ustilago maydis (DC.) Corda	*Ustilaginaceae; Ustilaginales*	AF038826

Morphological observation

The cultivated strains were examined frequently over 12 weeks to observe the growth of the colony. When ascomata were visible, daily observation was carried out to distinguish the different stages of ascomatal development. For ascomatal initial stages, minute tufts of hyphae were removed and placed in a drop of water on a slide for microscopic observation. For later ascomatal development stages, the ascomata were removed under the dissecting microscope and sectioned by using a freezing microtome or dissected on the slide to spread the asci and ascospores. Most preparations were mounted in water and observed immediately under the microscope. Some of the slides were stained with cotton blue in lactic acid to preserve the structure for later observation and photographing.

Results

Molecular analysis

The complete sequences of the ITS region of the strains CS20, CS22, CS6-61 and CS18 were 521–543 bp long. The ITS sequences from CS20, CS22 and CS6-61 were identical and different from that of CS18, which was identified as *C. sinensis*. The sequences have been submitted to GenBank with accession numbers from AY608922 to AY608925.

The sequences obtained from the BLAST search could be divided into several groups based on the taxonomic position of the fungi. They were named *Pesudogymnoascus* and its related fungi, including *P. roseus*, *Geomyces*, *Gymnostellatospora* Udagawa et al. and *Chrysosporium* Corda. The majority of the sequences were of discomycetes, including *Helotiales* and *Rhytismatales*, and of other ascomycetes, including *Dothideales* and *Erysiphales*. The sequences selected for analysis represented the major groups found in the BLAST search. To clarify the systematic relationship of CS20, CS22 and CS6-61 with *Onygenales* species, sequences of *Gymnoascus*, *Myxotrichum* Kunze, *Amauroascus*, *Arthroderma* and *Ajellomyces* were included in the analysis.

A total of 675 bp of the ITS regions was used in the analyses. Among the nucleotides, 185 were constant. Of the remaining variable bases, 329 were potentially parsimony informative. Ten most parsimonious trees were obtained with this alignment. One of them is shown in Fig. 1. The sequences formed four groups marked as pseudogymnoascean, discomycetous, pyrenomycetous and onygenalean according to the species within each group. The other nine trees differed from Fig. 1 in the positions of AF081431-*Pseudogymnoascus roseus* and AF062818-*Gymnostellatospora japonica* within the pseudogymnoascean group; the position of AF203469-*Elytroderma deformans* being a sister group to both the pseudogymnoascean group and the major clade of the discomycetous group in some other trees; and the positions of AF335454-*Bisporella citrina* and U72611-*Gelatinipulvinella astraeicola* (as '*astraoeca*' in Genbank) within the discomycetous group.

The pseudogymnoascean group comprised sequences of *P. roseus*, *Geomyces* and *Gymnostellatospora* and was supported by bootstrap analysis at 88% (Fig. 1). The sequences of CS20, CS22, CS6-61 and of AF062819, named *P. roseus* in GenBank, were identical. AF081431-*P. roseus* is an incomplete ITS sequence containing only ITS2 and partial 5.8S gene, almost identical to the same part of ITS sequences of AF062819-*P. roseus*, CS20, CS22 and CS6-61. Although it was shown with no change from others of these sequences in Fig. 1, it was sometimes placed as a sister group to AF062819-*P. roseus*, CS20, CS22 and CS6-61 in the other parsimony trees. The grouping of these sequences in a terminal clade received 92% bootstrap support. Sequences of AJ509872 and AF015789, both named as *Geomyces pannorum* in Genbank, grouped together with 83% bootstrap support. They formed a clade with the *P. roseus* sequences having bootstrap support of 74%. The other two sequences of *Geomyces*, AF307760-*G. pannorum* and AJ390390-*G. asperulatus*, are almost identical with only two nucleotide substitutions. The two sequences clustered with AF062818-*Gymnostellatospora japonica* having 69% support in Fig. 1, but the latter was grouped with the *P. roseus* and *Geomyces pannorum* clade in some other trees. Several records of named *Geomyces pannorum* in GenBank (accession numbers from AJ509866 to AJ509871) have similar sequences to AJ509872 and AF015789 and were represented by the latter two in this analysis.

The discomycetous group mainly contained *Helotiales* and *Rhytismatales*. *Myxotrichum arcticum* (*Myxotrichaceae*) and *Erysiphe cichoracearum* (*Erysiphaceae*) were also included in this group. The discomycetous group was the sister group to the pseudogymnoascean group, but it was polyphyletic because one of the discomycetes, *Elytroderma deformans*, was placed as an immediate sister group to the pseudogymnoascean group (Fig. 1). In fact, the pseudogymnoascean group is imbedded within the discomycete taxa.

Neurospora tetrasperma (AF388929) and *Cordyceps sinensis* (CS18) were clustered together to form the pyrenomycetous group, which was supported by 100% in bootstrap analysis. The pyrenomycetous group is the sister group to the clade containing pseudogymnoascean and discomycetous groups.

Five species from four families of *Onygenales* were clustered together forming the onygenalean group. The support for this group was very strong, reaching 95% in bootstrap analysis although they demonstrated many variations in ITS sequences.

Morphological descriptions

Morphological characters of the strains, including colony, anamorph and teleomorph, were observed and described from culture. The developmental sequence of ascomata is described and illustrated in detail to demonstrate the formation of a massive ascoma.

Culture: Isolates of CS20, CS22 and CS6-61on bran-agar medium formed white colonies with thick aerial mycelium covering the sclerotium tissue. Colonies of the cultures were flocculant with woolly aerial hyphae, white at first but later becoming pinkish brown to purple, with pigmentation varying in the same colony. Reverse of the colony was red-brown to purplish brown. Ascomata appear in 6–8 weeks. Mature ascomata were pale yellowish-brown to pinkish brown and were either scattered upon the surface of the colony or aggregated into dense clusters. Finally the woolly aerial hyphae disappear and the colonies are covered with clay-pink, farinaceous granules.

Microscopic observation: Vegetative hyphae were hyaline, 1.0–2.5 μm diam. Ascomata were discrete or confluent, globose to subglobose, 52–320 μm diam., at first white, finally pale pink to pinkish brown under the dissecting microscope. The outer part of the ascoma formed a defined layer of hyphae, which was usually regarded as a peridium. The peridium was composed of pale yellow to yellow-brown, septate, thick-walled hyphae, 2.0–3.0 μm diam., sometimes thickening at the node reaching 3.5 μm diam. Thin-walled hyphae arising from the thick-walled hyphae in the peridium were regarded as appendages, which were simple and up to 40 μm long. Asci were hyaline,

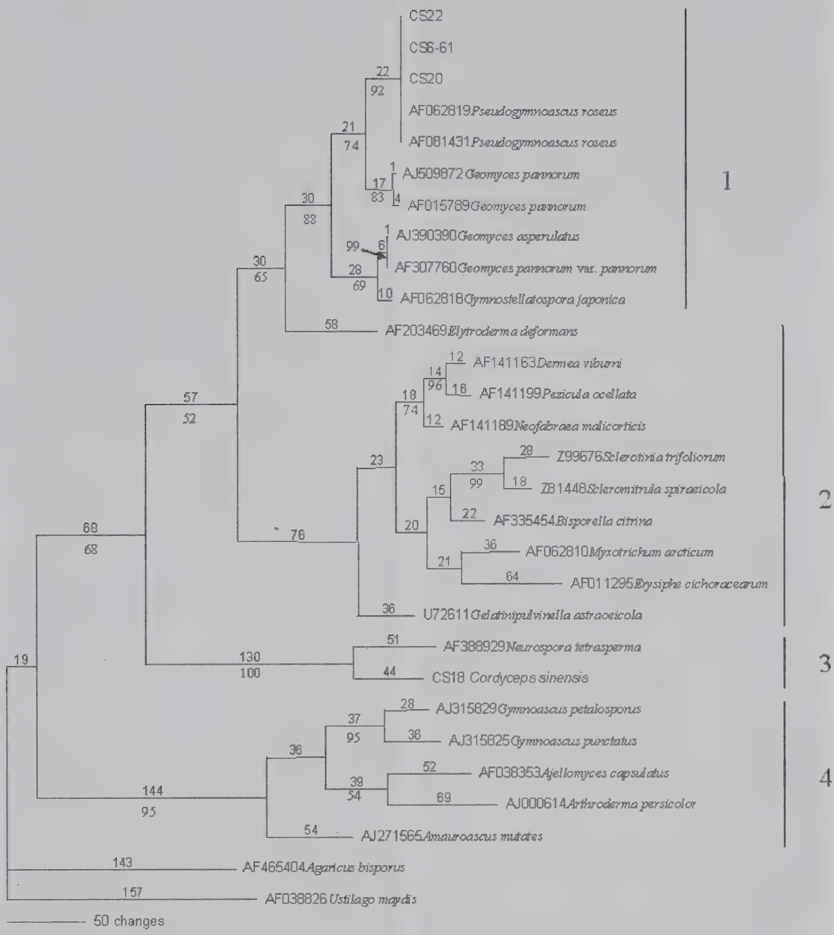
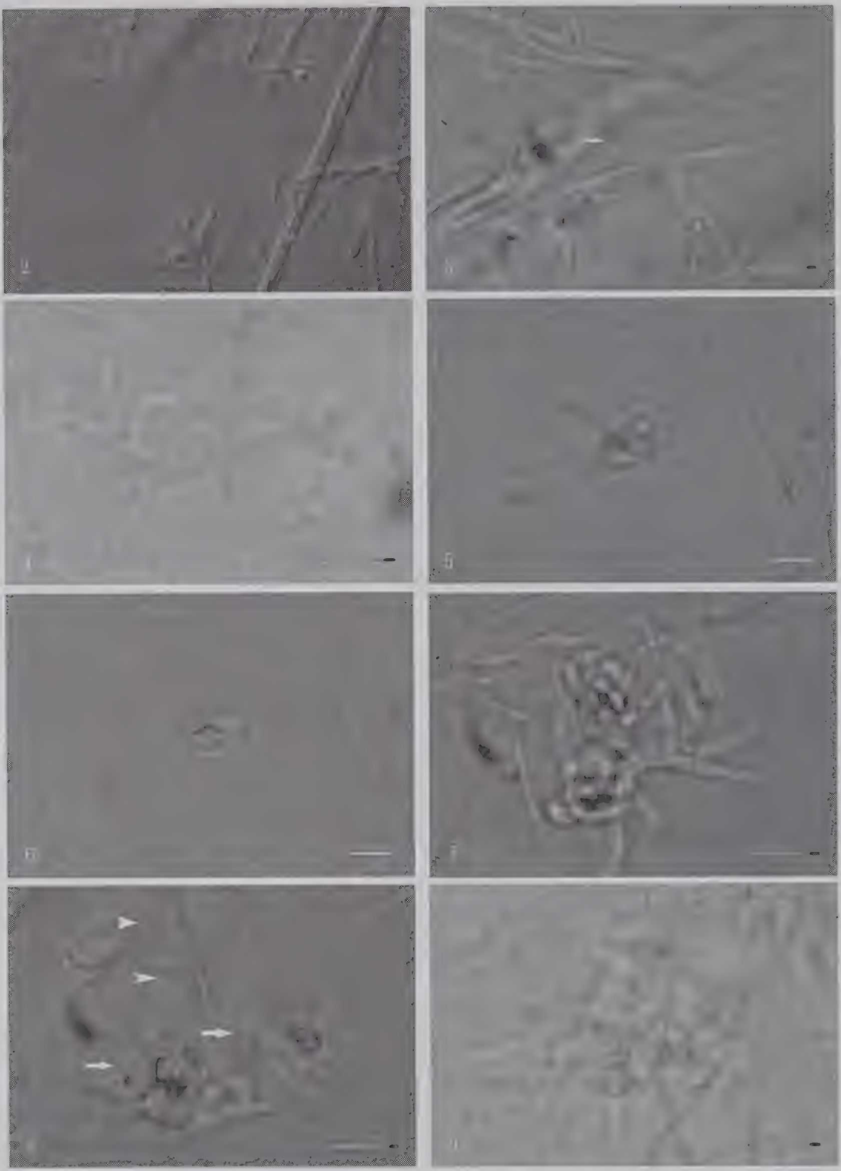

CS22
CS6-61
CS20
AF062819 Pseudogymnoascus roseus
AF081431 Pseudogymnoascus roseus
AJ509872 Geomyces pannorum
AF015789 Geomyces pannorum
AJ390390 Geomyces asperulatus
AF307760 Geomyces pannorum var. pannorum
AF062818 Gymnostellatospora japonica
AF203469 Elytroderma deformans
AF141163 Dermea viburni
AF141199 Pezicula ocellata
AF141189 Neofabraea malicorticis
Z99676 Sclerotinia trifoliorum
Z81448 Scleromitrula spiraeicola
AF335454 Bisporella citrina
AF062810 Myxotrichum arcticum
AF011295 Erysiphe cichoracearum
U72611 Gelatinipulvinella astraeicola
AF388929 Neurospora tetrasperma
CS18 Cordyceps sinensis
AJ315829 Gymnoascus petalosporus
AJ315825 Gymnoascus punctatus
AF038353 Ajellomyces capsulatus
AJ000614 Arthroderma persicolor
AJ271565 Amauroascus mutates
AF465404 Agaricus bisporus
AF038826 Ustilago maydis
1
2
3
4
50 changes

Fig. 1. One of the ten most parsimonious trees obtained from the analysis of nucleotide sequences of ITS regions (nrDNA). The upper and lower numbers on each branch denote the number of estimated substitutions and the percentage of bootstrap replicates respectively. Only bootstraps higher than 50% are shown. The length of the tree is 1607 steps, with consistency index=0.5083 and retention index=0.5401. Group 1=pseudogymnoascean, 2=discomycetous, 3=pyrenomycetous and 4=onygenalean.

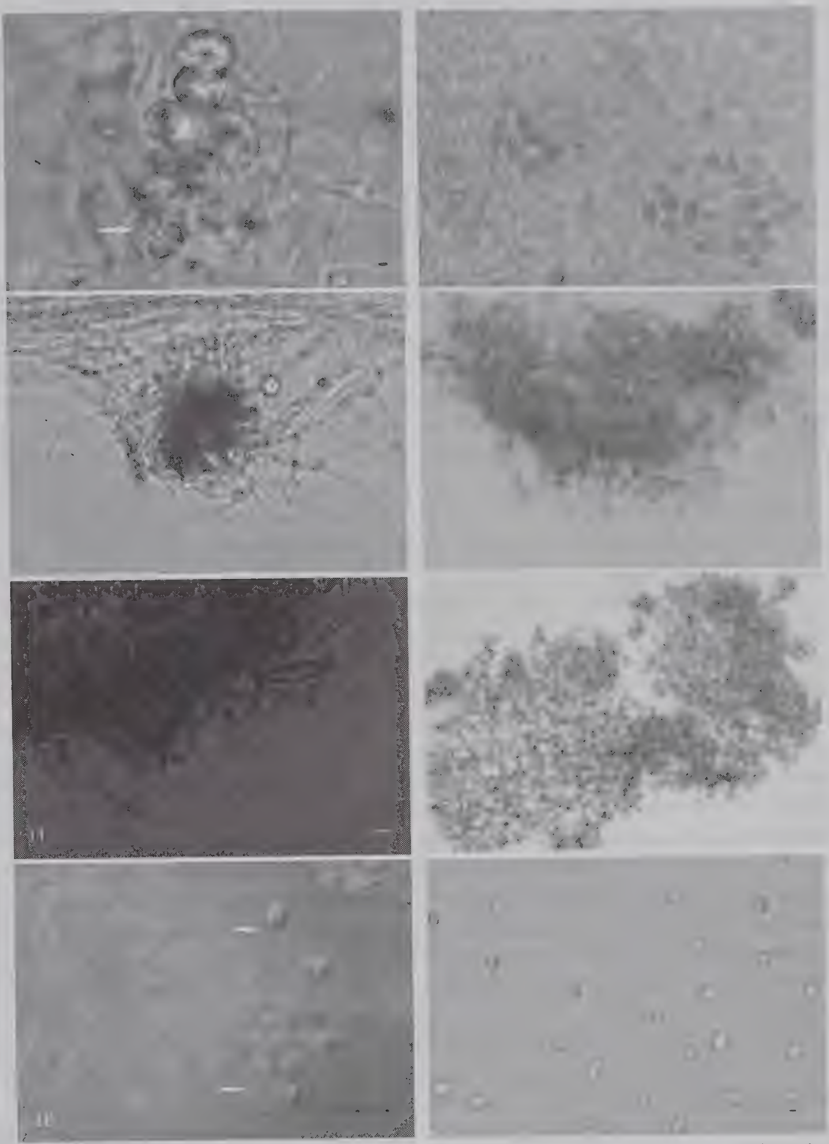
subglobose to oval, stalked, 8-spored, (6.0–) 7.0–9.0 μm diam. Ascospores were hyaline, ellipsoid to fusoid-ellipsoid or fusoid, pale olivaceous to pink, smooth, yellowish brown in mass, 3.5–5.5×1.8–3.0 μm.

Anamorph: Hyphae were hyaline, smooth and thin-walled, 0.8–2.5 μm wide. Conidiophores were hyaline and dendroid, or sometimes absent. Conidia formed terminally or were intercalary, thin-walled, smooth, 3.0–6.0×2.0–3.5 μm. The terminal conidia had a truncate base (about 1.8 μm wide) and were cuneiform, ellipsoid or ampullaceous. The intercalary conidia were barrel-shaped. Numerous conidia sometimes



Figs 2–9. *Pseudogymnoascus roseus.* **Fig. 2.** Anamorphic stage. Terminal and intercalary conidia were borne on dendroid conidiophores. **Fig. 3.** Ascoma initial. The ascoma initial arose as a short branch (arrow) from a vegetative hypha. **Fig. 4.** Two pairs of adjacent ascoma initials. The right two initials curved towards each other whilst the left two curved away to the opposite direction. **Fig. 5.** Ascoma initial coil. The other part of the initial is out of focus. **Fig. 6.** The same ascoma initial in Fig. 5 observed at a different focus. Hyphal branches emerge from the coil. **Fig. 7.** Close-up of two ascoma initials. The initials have complex coiling hyphae and branches. **Fig. 8.** Multiple ascoma initials from one site. Two tight ascoma initial coils (arrow heads) and two initial branches (arrows) are visible. **Fig. 9.** Young asci produced in groups. There is no ascospore delimitation at this stage.

Bar=10 μm, except for Fig. 2, where Bar=20 μm.



Figs 10–17. *Pseudogymnoascus roseus.* **Fig. 10.** Young ascospores formed within asci. Ascospores (arrow) are clearly visible at this stage and there is no envelopment of the asci, which were produced from the same ascoma initial. **Fig. 11.** Groups of asci. The groups of asci lack a defined envelope separating them from the surrounding hyphae and may merge together to form an ascus aggregation. Ascospores are visible within asci. **Fig. 12.** A small and immature globose ascoma. There is a released ascus to the upper right of the ascoma. **Fig. 13.** Part of a section of an ascoma. A large number of asci are enwrapped by a network of thick-walled hyphae (peridium). **Fig. 14.** Close-up of peridium in a section of an ascoma. The peridium hyphae are thick-walled with many branches. **Fig. 15.** Dissection of an ascoma. The asci in the ascoma are released at the upper right from the peridium, which is at the lower left. **Fig. 16.** Dissection of an ascoma. Asci are formed on stalks (arrow). **Fig. 17.** Released ascospores.
Bar=10 μm in Figs 10–12, 14 and 17; Bar=20 μm in Fig. 16 and Bar=30 μm in Fig. 13.

aggregated together to form a subglobose conglobation. If the strains were kept for long enough, ascomata of *P. roseus* appeared and the colonies became pinkish coloured in 6–8 weeks.

Development of ascomata: Ascomatal initials are scattered among, and formed from, vegetative hyphae. Initially, ascomatal initial branches, usually 2.5 μm diam. wide, arise from vegetative hyphae. The short branch with thicker cytoplasm than surrounding vegetative hyphae (Fig. 3) was the earliest stage of ascomatal initial observed. As ascomatal initials grew, they became curved. Adjacent ascomatal initials might sometimes grow toward each other or apart from each other (Fig. 4). Figure 4 shows two pairs of adjacent initials: the right two initials curved towards each other while the left two curved away to the opposite direction. The conjunction of two initials and differentiation of gametangia were not detected. Ascomatal initials continued to grow and formed coils (Figs 5, 6). Figures 5 and 6 show an ascomatal initial coil observed at different focuses. Some thick branch structure emerged from the coil. The initials coiled further to form helical loops (Figs 5–8). Several initials arising nearby were at the same or different developing stages (Figs 7, 8). Figure 8 shows two tightly coiled ascomatal initials (arrow heads) and two ascomatal initial branches (arrows) which arose from the same hypha. Figures 5–8 also show irregular branches stretched out from the coiled initials. These branches can continue to grow and form new coils, or interweave to form an enveloping network in the later stages.

As the ascomatal initial develops, asci are produced. Asci are globose and produced in groups (Fig. 9). Asci begin to enlarge and ascospores are delimitated and form inside the asci (Figs 10, 11). The asci aggregated to form large structures without any defined envelope separating them from the surrounding hyphal components (Figs 10, 11). Figure 11 demonstrates groups of asci can be produced close to each other, and asci from different initials can result in single ascus aggregations. As the number of asci increases, ascomata also increase in size and appear as dark spheres under the microscope owing to the thickness preventing light transmission (Fig. 12). The early ascomata appear as small white points attached to white vegetative hyphae under the dissecting microscope.

As ascus aggregation develops and merges from different ascomatal initials, a network of thick-walled hyphae also envelops the asci. The thick-walled hyphae interweave to form a loose network, which is pale yellow to yellow in colour. Pigmentation of these hyphae increases as the ascomata enlarge. Eventually, a specified layer of the hyphal network, i.e. the peridium, is formed. Figures 13 and 14 are sections of ascomata showing a large number of asci surrounded by the hyphal network (peridium). The peridial network comprises thick-walled hyphae which are richly branched and anastomosed (Figs 14, 15). Some thin-walled, short branches from the hyphal network extend from the globose ascoma. Figure 15 demonstrates that the peridium is composed of loosely interwoven hyphae, which forms a net enveloping the densely packed asci. The whole ascoma appeared dark coloured under the transmission microscope, but as spheres with some shades of pink to pale purple under the dissecting microscope.

Figure 16 shows the attachment of asci to hyphal stalks squeezed out of an ascoma which had a hyphal network (peridium) enveloping the ascus aggregation. When the ascoma matures, a large number of ascospores are released from asci spreading out of the ascoma and among the hyphal network. The ascospores were fusoid to ellipsoid (Fig. 17).

Discussion

The sequence comparison revealed that the ITS sequences of CS20, CS22 and CS6-61 were identical to AF062819-*Pseudogymnoascus roseus* in GenBank. The DNA sequence analysis showed that these sequences and AF081431-*P. roseus*, the partial sequence of ITS region included in this study, formed a unique terminal group. The strains CS20, CS22 and CS6-61 should be regarded as the same species as AF062819-*P. roseus*, which was submitted by Hambleton et al. in 1998 from strain UAMH 9163, isolated from roots of *Abies lasiocarpa* (Hook.) Nutt. in Alberta, Canada.

The morphological characters of CS20, CS22 and CS6-61 matched those described for *P. roseus* by Cejp & Milko (1966), Orr (1979) and Currah (1985). A reference collection of *P. roseus* deposited at K (K(M) 116466: *Pseudogymnoascus roseus*, isolated from *Eucalyptus*, Australia, G. Johnson, 1975) was also examined for confirmation and they showed the same morphological features. Based on the molecular and morphological data, CS20, CS22 and CS6-61 were identified as *P. roseus*. The strains also exhibit an anamorphic stage with dendroid conidiophores, and cuneiform to ellipsoid and terminal or intercalary conidia. Compared with the morphological characters of *Geomyces pannorum* var. *vinaceus* (Sigler & Carmichael 1976, Oorschot 1980), the anamorphic stage of these strains should be classified in the genus *Geomyces* and might be the same as *G. pannorum* var. *vinaceus*.

The two *G. pannorum* sequences (AF509872 and AF015789) demonstrated only a few variations from that of *P. roseus* and formed a clade with *P. roseus* in the ITS analysis. This result suggested that the two strains of *G. pannorum* were likely to be related to *P. roseus*. The sequence variation between *G. pannorum* and *P. roseus* was nearly the same as within the *G. pannorum* clade.

Four sequences of *Geomyces*, i.e. three *G. pannorum* and one *G. asperulatus*, were separated into two clades. It is possible that more than one taxon has been involved in the strains named as *G. pannorum*. The sequence of AF307760-*G. pannorum* var. *pannorum* is closer to AJ390390-*G. asperulatus* than to the other two *G. pannorum* isolates. *Geomyces asperulatus* and *G. pannorum* are similar in morphology. *Geomyces asperulatus* has hyaline, narrow, sometimes branching conidiophores 0.5–1.0 μm long, and yellow, barrel-shaped (or cuneiform if terminal), long-chained arthroconidia whist *G. pannorum* differs from *G. asperulatus* in forming conidia in shorter chains and in having aleurioconidia formed laterally on the hypha. It is not easy to distinguish the two taxa based on morphology. It appears that molecular systematic analysis based on the DNA sequence data may be a reliable method to avoid misidentification. *Gymnostellatospora japonica* was clustered with two of the four *Geomyces* sequences (AJ390390 and AF307760), and is possibly related to *P. roseus* and *Geomyces* species, but they may not be the same species judged from the sequence variations and from the parsimony analysis. *Gymnostellatospora* was published and placed in *Myxotrichaceae* by Udagawa et al. (1993), based on gross morphological characteristics. Species of *Gymnostellatospora* possess yellowish or reddish brown ascomata; hyphal network of peridium; indistinct 'appendages'; 8-spored, globose or ovoid, evanescent asci; and fusoid, hyaline to pale yellow ascospores (Udagawa et al. 1993). These features are similar to those of *Pseudogymnoascus*. However, the peridial hyphae of *Gymnostellatospora* are incompositoperidium-type (see Currah 1985) at maturity and the ascospore wall has narrow longitudinal crests and a convex surface, which is clearly different from that of *Pseudogymnoascus*. If the two genera are more widely sampled for investigation of molecular sytematics, it may be possible to find relationships between them.

The systematic position of the genera *Gymnostellatospora*, *Myxotrichum* and *Pseudogymnoascus* were classified in the same family, *Myxotrichaceae*. Members of these genera are similar in morphology, having brown shaded ascomata; hyaline, globose or subglobose asci; and hyaline, ellipsoid or fusoid ascospores. In the present ITS sequence analysis, *Myxotrichum arcticum* was distantly related to *P. roseus* and *Gymnostellatospora japonica*. *Myxotrichum arcticum* was placed in the discomycetous group, and *P. roseus* and *Gymnostellatospora japonica* formed a separate clade with the anamorphic *Geomyces*. Members of *Myxotrichaceae* in this analysis were not in a monophyletic group, as speculated by Currah (1994) based on ascospore morphology. They were separated and embedded among discomycete taxa. Based on 18S rDNA analysis, Sugiyama et al. (1999) described two separate lineages within *Myxotrichaceae*, in which *Myxotrichum* was separated from *Pseudogymnoascus*. Hambleton et al. (1998) also reported that *Myxotricum* and *Byssoascus* Arx diverged significantly from *Gymnostellatospora japonica* and *P. roseus* in ITS sequence analysis. The result of the present study coincides with those reports by Sugiyama et al. (1999) and Hambleton et al. (1998). Although the close relationship between members of *Myxotrichaceae* and discomycetes has been revealed in molecular studies, their ascomatal structure and morphology differ remarkably. Asci of discomycetes are formed from a hymenium within the apothecium, but a hymenium or apothecium are absent in *P. roseus* and other species of *Myxotrichaceae*.

Pseudogymnoascus was formerly placed in *Onygenales* (e.g. Currah 1985, 1988). The onygenalean fungi produce evanescent asci in the mycelium or cleistothecial ascomata, and unicellular ascospores. To confirm their molecular relationship, several ITS sequences of onygenalean fungi were included in this analysis. The sequences of onygenalean fungi greatly diverged from those of *P. roseus*, *Geomyces pannorum* and other selected *Myxotrichaceae*. *Pseudogymnoascus roseus* and *Myxotrichum arcticum* are separated from the onygenalean group by the pyrenomycetous group, and closely related to the discomycetes. The results of the present study reveal that the onygenalean group is distantly related to the *Myxotrichaceae* in ITS sequence and shows the independent evolutionary line of *P. roseus* and *Myxotrichum* from other onygenalean taxa as suggested by Currah (1994). Recent molecular systematic studies (Sugiyama et al. 1999, Mori et al. 2000) based on a different region of rDNA also obtained the same results.

The onygenalean fungi were rather isolated from other ascomycetes tested. The discomycete and pyrenomycete species were more closely related to each other than to the onygenalean fungi (Fig. 1). Furthermore, many variations in ITS sequences were also observed among the onygenalean fungi. It seems that there is a complex relationship in *Onygenales* and great divergence may exist within the order.

Morphologically, *Pseudogymnoascus* is similar to members of *Onygenales* in many aspects. The ascomatal stucture and brown colour of the reticulate hyphal network (peridium) in *Pseudogymnoascus* is very close to that of *Gymnoascus*. The characters of the peridium were very much emphasised in previous classifications and *Pseudogymnoascus* was considered to be related to *Gymnoascus*, which possesses oblate ascospores with an acute rim. *Pseudogymnoascus* differs from *Gymnoascus* only in the absence of peridial appendages and ascospore morphology. Currah (1985) noted that characters of ascospore morphology, enzymatic capacities, and nature and the occurrence of anamorphs provide significant information for classification. Based on ascospore morphology and cellulolytic capacities, Currah (1985) placed *Pseudogymnoascus* in *Myxotrichaceae* and *Gymnoascus* in *Gymnoascaceae*, thus separating these two genera in different families. Molecular investigations (Sugiyama et al. 1999, Mori et al. 2000, and this study) also consider that *Pseudogymnoascus* is widely separated from members of *Gymnoascus* and other onygenalean fungi.

Different peridial types evolved within the prototunicate ascomycetes in response to selective pressures affecting spore dispersal from enclosed areas (Currah, 1994). The loose hyphal network of the peridium exposes the asci and ascospores. Along with the evanescent asci, this may contribute to the release and dispersal of ascospores and increase the chance of propagation. The similar peridium occurring in quite different lineages of genera may be the result of convergent evolution.

The close relationship of *P. roseus* with the discomycetes has been suggested by molecular investigations (Sugiyama et al. 1999, Mori et al. 2000, and this study). *Pseudogymnoascus roseus* and other members of *Myxotrichaceae* share some characters in common: unicellular ascospores; scattered (without a hymenium), thin-walled and evanescent asci; cleistothecial ascomata; and ascomatal peridium of a thin hyphal weft. These characters are very different from those of typical discomycetes. Mori et al. (2000) considered that the close relationship between *Erysiphales*, *Myxotrichaceae* and some discomycetous fungi (mainly *Helotiales* and *Thelebolaceae*) suggested a novel evolutionary pathway from cleistothecial discomycetous fungi to *Erysiphales* and *Myxotrichaceae*. The present study also revealed a close relationship between *P. roseus*, other members of *Myxotrichaceae* and discomycete fungi.

Pseudogymnoascus roseus shares a similar structure of ascoma and peridium with members of *Onygenales*. The ascomatal structure and loose hyphal network of the peridium found in *P. roseus* and other species of *Myxotrichaceae* may have been reversed from the advanced apothecium to primitive ascoma in *Onygenales*, if the latter is considered to have diverged earlier in the ascomycetes. This reversion may also have occurred more then once (at least in the pseudogymnoascean group and *Myxotrichum arcticum* (Fig. 1) in the course of evolution.

Based on the observation of ascomatal development in this study, the formation of ascoma in *P. roseus* was initiated from short branches of vegetative hyphae. Currah (1985) generalised that the fertile ascomata of *Onygenales* were initiated by the formation of paired gametangial hyphae. Ascomatal development in *P. roseus* may be similar to that in *Onygenales*, but the microscopic observation made in this study did not detect the contact of two gametangia and the migration of nuclei or cytoplasm. The structure of hyphae in the ascomatal initial coils was complex and it proved difficult to distinguish their constituents under the light microscope.

Several ascomatal initials often appeared very close to each other from the same site in the colony of *P. roseus*. Asci from one or several initials might grow together without a defined envelope at early stages. Many asci grouped to form a large globose aggregation. A loose weft of hyphae (peridium) developed later, enveloping the aggregation. From the observation in this study, a large ascoma should be regarded as the ascus aggregation from several different ascomatal initials because it occupied a large space covering the area where different initials were formed. The peridium is only a loose network of thick-walled hyphae, and was termed a 'reticuloperidium' by Currah (1985). Like the ascoma, the peridium may also come from several different ascomatal initials. Although initial stages of ascomatal development and the structure of mature ascomata have been described (Cejp & Milko 1966, Orr 1979, Currah 1985), detailed observation of ascomatal formation has not been reported elsewhere. It is revealed for the first time that the ascomata in *Pseudogymnoascus* are formed by the aggregation of asci.

The morphology of ascospores and of peridial appendages are important taxonomic characters in recognising species of *Pseudogymnoascus*. The ascospores are generally considered smooth and the appendages not distinct in *Pseudogymnoascus*. Two species of *Pseudogymnoascus* have rather different features from *P. roseus*: *P. dendroideus* has ramified appendages and striated ascospores with slight thickening at the equator (Locquin-Linard 1982) and *P. alpinus* has navicular-fusoid ascospores, and branched and recurved appendages (Müller & Arx 1982). Either the taxonomic position of these two species is in need of reconsideration or the generic concept of *Pseudogymnoascus* must be revised. The species that Lumley et al. (2000) described as '*Pseudogymnoascus* sp.' also requires further investigation because the longitudinal sigmoid crest on the ascospores is not characteristic of *Pseudogymnoascus*. Molecular research may elucidate the relationships of these morphologically different fungi.

Pseudogymnoascus roseus is distributed worldwide and has often been isolated from alpine regions. It is interesting that the fungus was also isolated from sclerotia of *C. sinensis* in this study. Because of its medicinal value, *C. sinensis* has been the target of efforts to obtain pure isolates for massive production in industrial fermentation. Various fungi, about 22 names in 13 different genera, have been related in the literature to the anamorph of *C. sinensis*, either for isolates from material of the fungus or for postulated asexual states (Jiang & Yao 2002). Although many of the isolates are not true *C. sinensis*, they may have similar chemical properties and medicinal effects, e.g. *Paecilomyces sinensis* Q. T. Chen et al. and *Tolypocladium sinense* C. Lan Li (Gui & Chen 1983, Li 1988, Liu et al. 1991). Some of those isolates are even widely used in manufacture referring to the products of *C. sinensis* (Jiang & Yao 2002, 2003). It may be ascribed to the interaction of fungi or selective pressure in the same ecological environment that different fungi produce similar medicinal properties. Probably the strains of *Pseudogymnoascus roseus* were present in the alpine soil of the Tibetan Plateau and contaminated the *Cordyceps* isolates. As another micro-organism living in the same niche with *C. sinensis*, whether *P. roseus* has similar chemical properties that can be used for medicinal purpose may deserve further investigation. Currah (1985) suggested that the pale rose reticuloperidium and ascospores of *P. roseus* probably indicated a reliance on burrowing animals for dispersal. The host of *C. sinensis*, the caterpillar larvae of *Hepialus armoricanus* Oberthеir, living in the same microcosm may also be one of the animals helping the dispersal of *P. roseus* ascospores.

Acknowledgements

The authors are grateful to Drs B. M. Spooner and P. D. Bridge for serving as pre-submission reviewers and for their valuable comments and suggestions. This project is supported by the Chinese Academy of Sciences and the National Natural Science Foundation of China through the 'Key Research Direction of Renovation Program (KSCX2-SW-101C)' and the scheme of 'Introduction of Overseas Outstanding Talents', and 'National Science Fund for Distinguished Young Scholars' (30025002) respectively.

References

Alexopoulos CJ, Mims CW. 1979. Introductory Mycology. 3rd Edition. John Wiley & Sons, Inc., New York, USA.

Alexopoulos CJ, Blackwell M, Mims CW. 1996. Introductory Mycology. 4th Edition. John Wiley & Sons, Inc., New York, USA.

Altschul SF, Madden TL, Schäffer AA, Zhang J-H, Zhang Z, Miller W, Lipman DJ. 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Research 25: 3389–3402.

Arx, JA von. 1971. On *Arachniotus* and related genera of the *Gymnoascaceae*. Persoonia 6: 371–380.

Arx, JA von. 1974. The general of fungi sporulating in pure culture. 2nd Edition. J. Cramer, Vaduz, Germany.

Arx, JA von. 1981. The genera of fungi sporulating in pure culture. 3rd Edition. J. Cramer, Vaduz, Germany.

Arx, JA von. 1987. A re-evaluation of the *Eurotiales*. Persoonia 13: 273–300.

Benny GL, Kimbrough JW. 1980. A synopsis of the orders and families of plectomycetes with keys to genera. Mycotaxon 12: 1–91.

Bowman BH, Taylor JW. 1993. Molecular phylogny of pathogenic and non-pathogenic Onygenales. In: The Fungal Holomorph: mitotic, meiotic and pleomorphic speciation in fungal systematics (eds. D.R. Reynolds and J.W. Taylor) CAB Inernational, Wallingford, UK: 169–178.

Cejp K, Milko AA. 1966. Genus *Pseudogymnoascus* Raillo. (*Gymnoascaceae*). Céska Mykologica 20: 160–163.

Christensen M, Whittingham WF, Novak RI. 1962. The soil microfungi of wet-mesic forests in southern Wisconsin. Mycologia 54: 374–388.

Currah RS. 1985. Taxonomy of the *Onygenales*: *Arthrodermataceae*, *Gymnoascaceae*, *Myxotrichaceae* and *Onygenaceae*. Mycotaxon 24: 12–16.

Currah RS. 1988. An annotated key to the genera of the *Onygenales*. Systema Ascomycetum 7: 1–12.

Currah RS. 1994. Peridial morphology and evolution in the prototunicate ascomycetes. In Ascomycete systematics: problems and perspectives in the nineties (ed., D.L. Hawksworth). Plenum Press, New York, USA: 281–293.

Dalpé Y. 1989. Ericoid mycorrhizal fungi in the *Myxotrichaceae* and *Gymnoascaceae*. New Phytologist 113: 523–527.

Ellis MB, Ellis JP. 1988. Microfungi on Miscellaneous Substrates. An Identification Handbook. Croom Helm etc., London. UK.

Eriksson O, Hawksworth DL. 1986. Notes on ascomycete systematics. Systema Ascomycetum 5: 113–174.

Eriksson O, Hawksworth DL. 1993. Outline of the ascomycetes-1993. Systema Ascomycetum 12: 51–257.

Farrow WM. 1954. Tropical soil fungi. Mycologia 46: 632–646.

Felsenstein J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39: 783–791.

Gui X-M, Chen X-L. 1983. Research on chemical component of *Paecilomyces sinensis*. Bulletin of Chinese Materia Medica 8(4): 32. [in Chinese].

Hall TA. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series 41: 95–98.

Hambleton S, Egger KN, Currah RS. 1998. The genus *Oidiodendron*: species delimitation and phylogenetic relationships based on nuclear ribosomal DNA analysis. Mycologia 90: 854–869.

Ito T, Yokoyama T. 1985. Filamentous fungi collected in far eastern USSR. Institute for Fermentation, Osaka, Research Communications 12: 34–62.

Ito T, Yokoyama T. 1987. Descriptive catalogue of IFO fungus collection X. Institute for Fermentation, Osaka, Research Communications 13: 83–85.

Jiang Y, Yao Y-J. 2002. Names related to *Cordyceps sinensis* anamorph. Mycotaxon 84: 245–254.

Jiang Y, Yao Y-J. 2003. Anamorphic fungi related to *Cordyceps sinensis*. Mycosystema 22: 161–176. [in Chinese].

Kirk PM, Cannon PF, David JC and Stalpers JA. 2001. Dictionary of the Fungi. 9th Edition. CAB International, Wallingford, UK.

Kuehn HH. 1958. A preliminary survey of the *Gymnoascaceae*. I. Mycologia 50: 417–439.

LeClerc MC, Philippe H, Gueho E. 1994. Phylogeny of dermatophytes and dimorphic fungi based on large subunit ribosomal RNA sequence comparisons. Journal of Medical and Veterinary Mycology 32: 331–341.

Li Z-L. 1988. A study of *Tolypocladium sinense* C. L. Li sp. nov. and cyclosporin production. Acta Mycologica Sinica 7: 93–98. [in Chinese].

Liu Y-R, Wu C-Z, Li C-C. 1991. The anti-oxidization effect of *Paecilomyces sinensis*. Journal of Chinese Materia Medica 16: 240–242. [in Chinese].

Locquin-Linard M. 1982. *Pseudogymnoascus dendroideus* Locquin-Linard, nouvelle espece de *Gymnascale* (Ascomycetets) Coprophile d'afrique du nord. Cryptogamie Mycologie 3: 409–414.

Lumley TC, Abbott SP, Currah RS. 2000. Microscopic ascomycetes isolated from rotting wood in the boreal forest. Mycotaxon 74: 395–414.

Maddison DR. 1991. The discovery and importance of multiple islands of most-parsimonious trees. Systematic Zoology 40: 315–328.

Mori Y, Sato Y, Takamatsu S. 2000. Molecular phylogeny and rediation time of *Erysiphales* inferred from the nuclear ribosomal DNA sequences. Mycoscience 41: 437–447.

Müller E, Arx JA von. 1982. *Pseudogymnoascus alpinus*, nov. spec. Sydowia Annales Mycologici 35: 135–137.

Oorschot CAN van. 1980. A revision of *Chrysosporium* and allied genera. Studies in Mycology 20: 1–99.

Orr GF. 1979. The genus *Pseudogymnoascus*. Mycotaxon 8: 165–173.

Samson RA. 1972. Notes on *Pseudogymnoascus*, *Gymnoascus* and related genera. Acta Botanica Neerlandica 21: 517–527.

Siddiqi MA. 1964. Fungus flora of coffea arabica in Nyasaland. Transaction of British Mycological Society 47: 281–284.

Sigler L, Carmichael JW. 1976. Taxonomy of *Malbranchea* and some other Hyphomycetes with arthroconidia. Mycotaxon 4: 349–488.

Sigler L, Lumley TC, Currah RS. 2000. New species and records of saprophytic ascomycetes (*Myxotrichaceae*) from decaying logs in the boreal forest. Mycoscience 41: 495–502.

Sugiyama M, Mikawa T. 2001. Phylogenetic analysis of the non-pathogenic genus *Spiromastix* (*Onygenaceae*) and related onygenalean taxa based on LSU sequences. Mycoscience 42: 413–421.

Sugiyama M, Ohara A, Mikawa T. 1999. Molecular phylogeny of onygenalean fungi based on small subunit ribosomal DNA (SSU rDNA) sequences. Mycoscience 40: 251–258.

Swofford DL. 2001. PAUP: phylogenetic analysis using parsimony. Version 4.0b10. Sinauer Associate, Sunderland, Massachusetts, USA.

Swofford DL, Olsen L. 1990. Phylogeny reconstruction. In Molecular Systematics (eds. M. Hilis and C. Mortiz). Sinauer Associates, Sunderland, Massachusetts, USA: 411–501.

Tsuneda AA. 1982. Scanning electron-microscopy of *Pseudogymnoascus roseus*. Mycologia 74: 844–847.

Udagawa S, Uchiyama S. 1999. Taxonomic studies on new or critical fungi of non-pathogenic *Onygenales*. Mycoscience 40: 277–290.

Udagawa S, Uchiyama S, Kamiya S. 1993. *Gymnostellatospora*, a new genus of the *Myxotrichaceae*. Mycotaxon 52: 157–164.

White TJ, Bruns T, Lee S, Taylor JW. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In PCR Protocols, A Guide to Methods and Applications (eds. M.A. Innis, D.H. Gelfand, J.J. Sninsky and T.J. White). Academic Press, London, UK: 315–322

Xiao G, Berch SM. 1995. The ability of known ericoid mycorrhizal fungi to form mycorrhizae with *Gaultheria shallon*. Mycologia 87: 467–470.

Yao Y-J., Pegler DN, Chase MW. 1999. Application of ITS (nrDNA) sequences in the phylogenetic study of *Tyromyces* s. l. Mycological Research 103: 219–229.

Yokoyama T, Ito T, Yin Y-Q. 1989. Filamentous fungi isolated from soils in the XinJiang Uighur Autonomous Region, China. Institute for Fermentation, Osaka, Research Communications 14: 118–142.

MYCOTAXON

Volume 94, pp. 75–84 *October–December 2005*

Morphological and molecular characterization of the mycorrhizas of *Inocybe rufuloides* and *I. splendens*

Mirco Iotti, Mauro Marchetti,
Enrico Bonuso & Alessandra Zambonelli

zambonel@agrsci.unibo.it
Dipartimento di Protezione e Valorizzazione Agroalimentare
via Fanin 46, 40127 Bologna, Italy

Abstract—The mycorrhizas of *Inocybe rufuloides* on *Pinus pinea* and of *Inocybe splendens* on *Ostrya carpinifolia* were characterized by morphological and molecular methods. Molecular identification was performed by comparing the rDNA ITS sequences obtained from *Inocybe* fruiting bodies with those of the mycorrhizas collected from the same area. Morphological characterization was carried out by a simplified Agerer protocol. This study provides sets of characters which can be used in further studies for the identification of these two *Inocybe* species found contaminating *Tuber* infected plants in experimental truffières.

Key words—ectomycorrhizas, ribosomal DNA sequences, morphological description, truffle cultivation

Introduction

Ectomycorrhizas are dominant in forests of temperate and boreal regions of the Northern Hemisphere (Brundrett et al. 1996). It has been estimated that 6000 or more species of fungi form ectomycorrhizal associations with approximately 10% of the Angiosperms and many Gymnosperms (Trappe 1987). The ectomycorrhizal communities are dynamic and changes in their population structure occur depending on the age of the host plants, season and environmental factors (Molina et al. 1992, Deacon & Fleming 1992). In the past research into ectomycorrhizal fungal diversity were almost exclusively carried out by identifying and counting fruiting bodies that appeared above the ground or by inspecting the roots of trees and grouping their ectomycorrhizas into morphotypes (Selosse 2001). While the first method is relatively quick and easy the number of species of ectomycorrhizal fungi that fruit above the ground is generally considered to be a gross underestimate of the number of ectomycorrhizal fungi present in an environment (Yamada & Katsuya 2001, Selosse 2001). For example, ectomycorrhizal fungi such as *Cenococcum geophilum* and many members of *Telephoraceae,* which are some of the most common mycorrhizas (Richard et al. 2005, Horton & Bruns 2001), do not produce fruiting bodies or form cryptic fruiting structures. In contrast, although morphotyping of ectomycorrhizas give a better picture of fungal diversity and subtle changes that can occur during competition in ectomycorrhizal communities, it is often impossible to relate the morphotypes to a known fungal species (Horton & Bruns 2001).

The recent application of molecular methods has revolutionised our ability to identify ectomycorrhizal fungi. They are useful either for the certification of plants that have been inoculated with commercially valuable species such as *Tuber* and also allow us to carry out more ambitious studies on ectomycorrhizal community structures and competition within them (Amicucci et al. 2002, Dahlberg, 2001). A comparison of ITS sequences obtained, for example, from traditionally identified fruiting bodies with those obtained from mycorrhizas can provide a means for identifying the fungi that form mycorrhizas (Horton & Bruns 2001). However ITS sequences for many ectomycorrhizal fungi have yet to be determined and so their identification remains impossible.

The aim of the work presented here was to provide a set of molecular and morphological characters for the identification of *Inocybe rufuloides* and *Inocybe splendens* by ITS sequencing of the mycorrhizas and of their fruiting bodies and the morphological description of their mycorrhizas which were found as contaminants in cultivated truffières.

Materials and methods

Sampling

The fruiting bodies of the two *Inocybe* species used in this study were collected from two different experimental truffières of Emilia Romagna, Italy. *I. rufuloides* was found in October 2003 in a *Pinus pinea - Tuber borchii* productive truffière established in 1990. The plantation is located in Marina di Ravenna on the Adriatic coast (49° 30' 24" N, 17° 60' 70" E) on littoral sandy soil (Zambonelli et al., 2000) while *I. splendens* was collected in March 2004 in a *Tuber aestivum* truffière established in 1997 with *Corylus avellana, Ostrya carpinifolia* and *Quercus pubescens* infected seedlings in the park of S. Giulia, Palagano, Modena (44° 23' 50"N, 10° 39' 40"E, elevation 932 m) (Zambonelli et al. 2005). *I. rufuloides* was found near *P. pinea* in the *T. borchii* truffière and *I. splendens* close to the trunks of the *O. carpinifolia* seedlings in the *T. aestivum* truffière. Dried specimens of each species were deposited in the herbarium of the "Centro di Micologia" of Bologna (*I. rufuloides* n.1994, *I. splendens* n. 5033).

Root samples were also collected from the truffières under fruiting bodies by removing 6 cm diameter soil cores between 0-30 cm. The soil cores were stored overnight in refrigerator at 5 °C and the roots then removed by carefully washing over an 0.8 mm mesh sieve.

Morphological characterisation

The *I. rufuloides* and *I. splendens* basidiomes were identified on the basis of their macro- and microscopic morphological characters (Heim 1931; Kuyper 1986; Stangl 1989; Moser et al. 1994, 1996; Bon 1997; Franchi et al. 2001). The microscopic features of the basidiomes were examined in both fresh and dried material previous rehydrated in 10% KOH and stained with Congo red.

Samples of mycorrhizas putatively formed by *Inocybe* sp. were selected from the root samples of *Pinus pinea* and *Ostrya carpinifolia* on the basis of their morphological features following Agerer's descriptions of *Inocybe* mycorrhizas (Agerer 1987-2002). Their morphological features were then described using sets of characters suggested by Agerer (1987-2002, 1991). The colour of the fresh mycorrhizas was recorded under a stereomicroscope and compared with the Royal Botanic Garden colour identification chart (RBG chart) (1969). Mycorrhizal tips were then fixed in FAA bleached by heating to 90 °C in 10% w/v KOH and treated with a few drops of H_2O_2 for 20-30 seconds in order to better examine the anatomy of the mantle under the microscope (Giomaro et al. 2000). Ectomycorrhizal tips were fixed in glutaraldehyde (25%), embedded in Tissue Tek OCT (Sakura, Zoeterwounde, Netherlands) compound and then cut with a rotary cryomicrotome (Tissue Tek® II, Miles, Elkhart, IN, USA) (8-10 μm thickness). Serial sections were stained with cotton blue, mounted in lactic acid and observed under a ECLIPSE TE 2000-E microscope (Nikon, Tokyo, Japan).

Mean dimensions of fruiting body and mycorrhiza characters were determined using Axio Vision 2.05 software (Carl Zeiss Vision GmbH, Hallbergmoos, Germany) from images captured with a DXM1200F digital camera (Nikon, Tokyo, Japan) and standard deviations then calculated. Each biometrical character was the mean of at least 50 measurements.

Molecular characterisation

Molecular identification of the mycorrhizas and fruiting bodies was performed using sequence data of the ITS regions of the ribosomal DNA. Total genomic DNA was isolated by DNeasy® Plant Mini Kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions and then eluted in 50 μl of sterile water. Ten mycorrhizas of each morphotype were pooled and 100 μg of fruiting body tissue were used. ITS-1, 5.8S and ITS-2 regions were amplified in a 50 μl volume reaction containing 1-10 ng of genomic DNA, using the primers pair ITS1 and ITS4 (White et al., 1990) in a T gradient Thermal Cycler (BIOMETRA, Göttingen, Germany) according to Amicucci et al. (1996). PCRs were performed using 1.5 units of *Taq* DNA polymerase (Fermentas, Vilnius, Lithuania).

The amplified products were first purified by Gene Clean II kit (BIO 101, Vista, CA, USA) and then sequenced using both the primers mentioned above. Sequence reaction was performed using the ABI PRISM 3700 DNA Analyzer (Applied Biosystem, Foster City, CA, USA). The obtained ITS sequences of fruiting bodies and mycorrhizas were compared each other and with those on the GenBank (http://www.ncbi.nlm.nih.gov/BLAST/) and UNITE (http://unite.zbi.ee/analysis.php3) databases using BLASTN search (Altschul et al., 1997). The ITS sequences of the fruiting bodies obtained in this study have been deposited in GenBank with the following accession numbers: *I. rufuloides* (DQ067579) and *I. splendens* (DQ067580).

Results

Morphological characterization

Inocybe rufuloides Bon

The *I. rufuloides* basidiomes had pileus 15-35 mm broad, campanulate, conico-convex, then plano-convex, at maturity applanate, with or without umbo, with margin inflexed when young, dark brown, orange-brown, tomentose around disc, outwards fibrillose, with fibrils diverging and sometimes squamulose-subsquarrose. Lamellae were adnexed

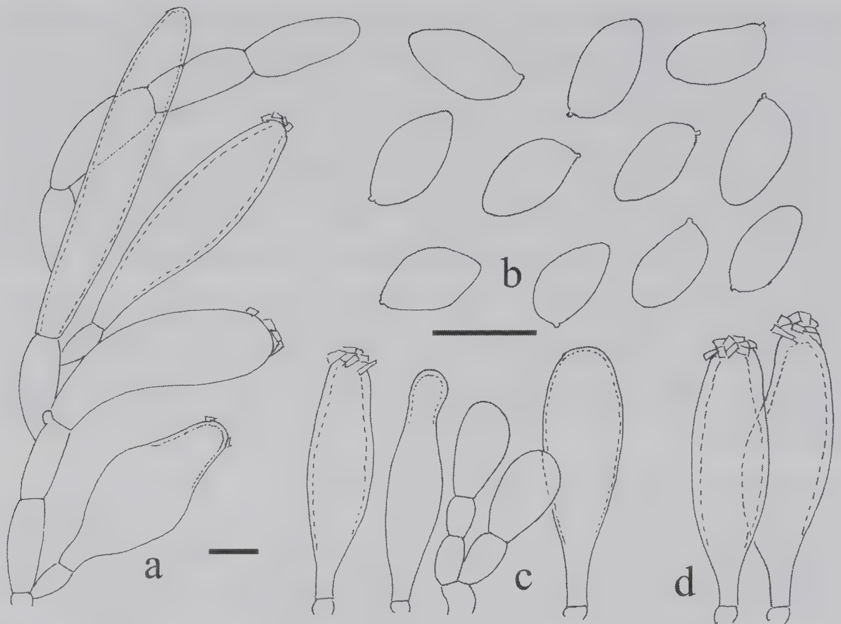


Fig. 1 – Caulocystidia (a), spores (b), cheilocystidia (c) and pleurocystidia (d) of *I. rufuloides* (Bars = 10 µm).

to adnate, L = 28-45, l = 1-3, moderately crowded, ventricose, 4-8 mm broad, pale greyish buff, then brown, dark brown, edge fimbriate, whitish. The stipe was 25-50 x 3-5 (6) mm, equal to clavate, subbulbous, brown to orange brown, extreme apex hairy-pruinose, near base whitish, downwards longitudinally white-fibrillose. The context was white in pileus, red-brown in stipe; smell and taste spermatic. The spores were (8) 10.5 ± 1.4 (13) x (4.7) 5.9 ± 0.7 (7) µm, Q = (1.5) 1.8 ± 0.2 (2.1), smooth, ovoid to amygdaliform, with subobtuse to indistinctly conical apex (Fig. 1b). The basidia were 28-38 x 9-12 µm, 2-4-spored. The hymenial cystidia were 50-70 x 10-20 µm, cylindrico-clavate, clavate, fusiform, lageniform, sometimes subcapitate, with a thick wall 1.5-2.5 µm, slightly yellow with ammoniac (Figs 1c & 1d). Caulocystidia were absent or present only at stipe apex (to 1/10th), similar to hymenial cystidia and mixed with cauloparacystidia (Fig. 1a), downwards are present caulocystidioid hairs.

I. rufuloides mycorrhizas were simple, ramified or with limited dichotomous branching involving a few lateral root tips, rarely coralloid. The unramified ends were short, straight or slightly twisted, 594.4 ± 167.3 µm long and 419.1 ± 79.0 µm in diameter. The structure of the surface was woolly with whitish emanating hyphae. The mycorrhizal tips were white (RBG chart n. 7) or greyish-cream (RBG chart n. 1) and orange-cream (RBG chart n. 44) at the base. When viewed with a microscope the mantle was plectenchymatous and had three distinct layers. The outermost was composed of partially bundled, loosely woven hyphae which were without a gelatinous matrix (Fig. 3a) whereas the middle and inner layers were made of tightly appressed hyphae (Fig. 3b & 3c). The mantle was 68.5

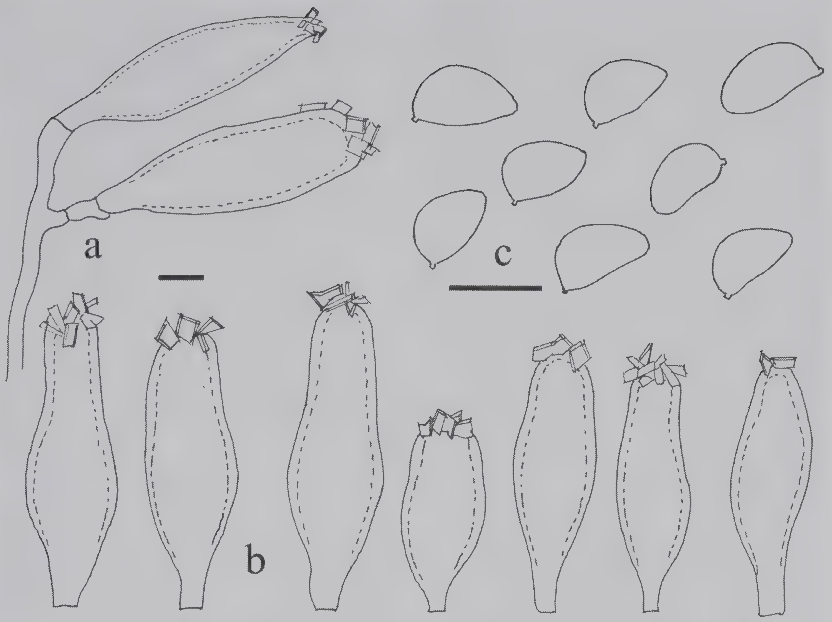


Fig. 2 - Caulocystidia (a), pleurocystidia (b) and spores (c) of *I. spendens* (Bars = 10 µm).

± 13.1 µm thick and the Hartig net was one, rarely two cells deep, 3.5 ± 1.1 µm thick and composed of a single hyphal row. In cross sections the mantle was composed of loosely arranged cells 4.7 ± 0.8 µm x 2.6 ± 0.5 µm in the external layers and 4.4 ± 0.9 µm x 2.8 ± 0.5 µm cells in the inner layer. The root cortical cells were tangentially oval 43.0 ± 9.1 µm x 19.6 ± 4.4 µm. The root cortical cells with Hartig net were radially oval 33.0 ± 7.4 µm x 18.0 ± 4.1 µm. In longitudinal section the mantle was composed of loosely arranged hyphal cells 5.4 ± 1.2 µm x 3.4 ± 0.9 µm in the external layer and of 5.0 ± 1.2 µm x 3.0 ± 0.6 µm in the inner layer. The shape of the root cortical cells was oval 58.3 ± 15.7 µm (tangentially) x 24.7 ± 5.5 µm. The root cortical cells adjacent to the Hartig net were 38.9 ± 9.8 µm (tangentially) x 19.7 ± 4.6 µm. The emanating hyphae (2.1 ± 0.4 µm thick) possessed clamp connections with a distinct hole and had a characteristically constricted fusion point between the arched part of the clamp and the parental hypha (Fig. 3d). Rhizomorphs and cystidia were absent.

Inocybe splendens R. Heim

I. splendens basidiomes had pileus 30-50 (70) mm broad, convex, soon plano-convex, finally applanate, with inflexed margin, later straight, often with conspicuous but low broad umbo, brownish-ochraceous to dark brown, sericeous-fibrillose, with fibrils not or diverging, sometimes radially rimulose. Lamellae were adnate to almost free, L=45-65, l=1-3, crowded, ventricose, 3-9 mm broad, whitish then greyish-yellow, finally yellow-brown, sometimes with olivaceous tinge, edge whitish, fimbriate. The stipe was 25-90

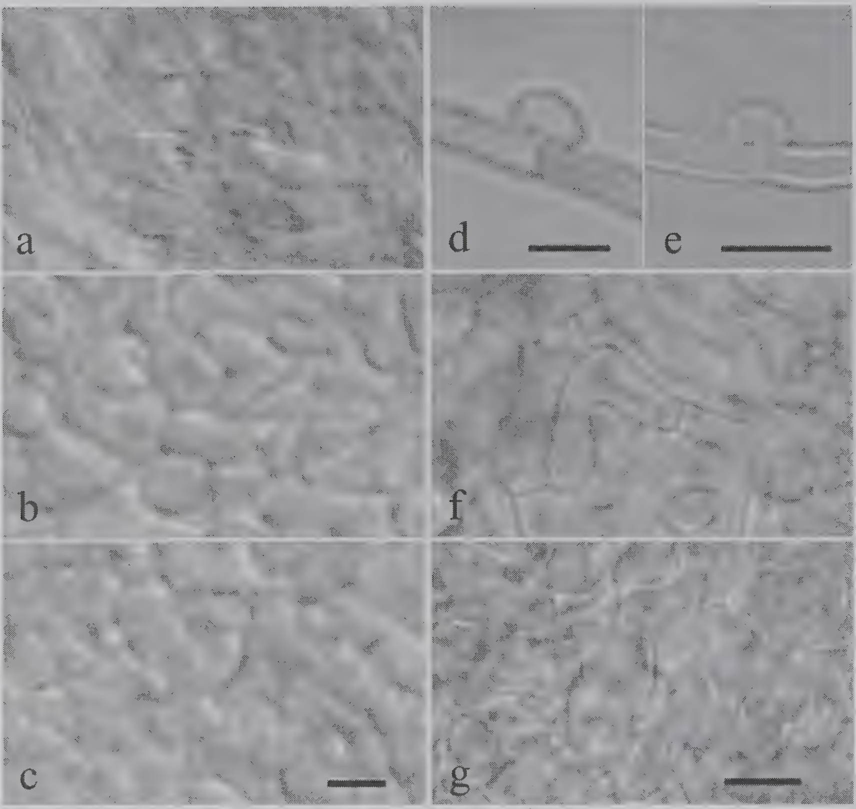


Fig. 3 – Mantle of *I. rufuloides* mycorrhizas (a external, b middle, c internal layers) and of *I. splendens* mycorrhizas (f external, g internal layers) under a light microscope. Clamp connections *of I. rufuloides* (d) and of *I. splendens* (e) (Bars = 5 μm).

x 6-15 mm, sometimes equal, but often marginately bulbous or subbulbous, whitish, finally ochraceous-yellow to pale brownish, pruinose all over. The context was white in pileus, ochraceous-yellow in stipe; smell subspermatic or as *Amanita phalloides,* taste not distinct. The spores were (8) 10.7 ± 1.5 (13) x (4.8) 5.9 ± 0.9 (7) μm, Q = 1.7 ± 0.2 (20), smooth, amygdaliform, with suprahilar depression, with subconical apex (Fig. 2c). The basidia were 26-40 x 8-12 μm, 4-spored. The hymenial cystidia were (45) 50-80 x 14-20 (27) μm, clavate, fusiform to utriform, sometimes sublageniform, with a thick wall 1.5-3.0 μm, colourless or slightly yellow with ammoniac, with crystalliferous at apex (Fig. 2b); paracystidia pyriform, thin-walled, colourless. The caulocystidia were similar to hymenial cystidia, descending almost to base of stipe, mixed with cauloparacystidia throughout (Fig. 2a).

I. splendens mycorrhizas were simple or with monopodial ramification with few lateral root tips. The unramified ends were straight or slightly twisted 965.2 ± 353.2 mm long and 222.4 ± 29.4 mm in diameter. The structure of the surface was woolly with whitish emanating hyphae. The colour of tips was white (RBG chart n. 7) or greyish-cream (RBG chart n. 1) and orange-cream at the base (RBG chart n. 44). Under a microscope the mantle was plectenchymatous, with two distinct layers, formed of closely appressed hyphae and without a gelatinous matrix (Fig. 3f & 3g). The thickness of the mantle was 27.2 ± 5.3 μm. The Hartig net was one, rarely two cells deep, 2.4 ± 0.5 μm thick, and one cell wide. In cross section the mantle was composed of appressed hyphal cells 4.2 ± 1.3 μm x 2.0 ± 0.6 μm in the external layer and of 4.1 ± 1.3 μm x 2.0 ± 0.7 μm in the inner layer. The root cortical cells were approximately tangentially oval 31.7 ± 6.1 μm x 20.9 ± 5.0 μm. The root cortical cells adjacent to the Hartig net were radially oval 27.7 ± 4.5 μm x 14.0 ± 2.7 μm. In longitudinal section the mantle was composed of appressed hyphal cells 4.4 ± 0.8 μm x 2.5 ± 0.6 μm in the external layer and 4.3 ± 1.0 μm x 2.5 ± 0.6 μm in the inner layer. The shape of the root cortical cells was oval 40.0 ± 7.5 μm (tangentially) x 19.9 ± 4.5 μm. The root cortical cells with Hartig net were 37.9 ± 7.4 μm (tangentially) x 13.1 ± 3.5 μm. The emanating hyphae (2.0 ± 0.3 μm thick) possessed clamp connections with a small hole and with a characteristically constricted fusion point where the arched part of the clamp met the parental hypha (Fig. 3e). Rhizomorphs and cystidia were lacking.

Molecular characterisation

The amplicons of *I. rufuloides* and *I. splendens* resulting from ITS1/ITS4 amplification showed length of 690 pb and 710 pb respectively. No differences were found between sequences obtained from mycorrhizas and from the respective fruiting body of each *Inocybe* species. The sequences resulting from ectomycorrhizae and fruiting body of *I. rufuloides* showed 93% level of similarity (510/544 nt) with a nearly complete ITS1-5.8S-ITS2 sequence of an uncultured ectomycorrhiza of *Inocybe* (accession number AY825514) described by Richard et al. (2005). Instead, the sequences resulting from ectomycorrhizae and fruiting body of *I. splendens* showed 90% level of similarity (580/640 nt) with a nearly complete ITS1-5.8S-ITS2 sequence of an uncultured fungus from ectomycorrhizal root isolate (accession number AY702725) described by Izzo et al. (2005). Lower similarities were obtained with the *Inocybe* sequences in the UNITE database.

Discussion

The molecular and morphological characterization of *I. rufuloides* and *I. splendens* mycorrhizas provide reliable instruments for the identification of these two fungi that are potential contaminants in natural and cultivated truffières.

Many species of ectomycorrhizal fungi are able to contaminate cultivated truffières such as less valuable *Tuber* species, *Cenococcum* spp., *Hymenogaster* spp., *Tomentella* spp., *Scleroderma* spp., *Hebeloma* spp., and several, as yet, unidentified fungi known only as morphotypes (Donnini & Bencivenga 1995, De Miguel & Sáez 2005). This replacement of inoculant fungi in truffières by more aggressive competing fungi is one of the most important problems during the cultivation of truffles (Sourzat 2001). These ectomycorrhizal species usually either have a broad range of adaptability to different ecological conditions or ecological requirements similar to cultivated truffles (Dalberg 2001, Giovannetti 1983). *I. rufuloides* is reported to grow in association with *Pinus* *maritima* on sandy soil along the Mediterranean coasts while *I. splendens* is associated with broad-leaf trees on calcareous sand and clay soils in Europe (Kuyper 1986). These are the same habitats where *T. borchii* and *T. aestivum* grow respectively. The discovery of *Inocybe* spp. in cultivated truffières and natural *Tuber magnatum* (Giovannetti 1983, Pirazzi 2001), *T. aestivum* (Chevalier & Frochot 1997) and *T. borchii* (Zambonelli et al. 2002) truffières, and their ecological requirements seems to confirm that members of this genus, might be important competitors.

The morphological characterization of *I. rufuloides* and *I. splendens* mycorrhizas confirms that some characters such as the plectenchymatus mantle structure, the form of the fusion point of clamp connections can be useful to distinguish the mycorrhizas formed by the species belonging to the genus *Inocybe* (Agerer 1987-2002). However, the only morphological characters don't allow identification at species level. Moreover, colour and form of the mycorrhizas can vary with their age; the form of the mantle cells, which is a quite important taxonomic character, can show some differences depending on the fungal strain and the host plant (Giomaro et al. 2000, 2002; Sisti et al. 2003).

The early identification of the presence of contaminant fungi in cultivated truffières offers growers the possibility to promptly introduce cultivation procedures that favour the establishment and development of *Tuber* ectomycorrhizas such as correct irrigation rates and mulching (Zambonelli et al. 2005).

Molecular analyses have allowed us to unequivocally identify *I. rufuloides* and *I. splendens* mycorrhizas by comparing their ITS sequences in the symbiotic and reproductive phases. The ITS sequence of *I. rufuloides* and *I. splendens* deposited in genbank will be a useful tool for other researchers who need to confirm the morphological identification of these fungi by the comparison of their sequences and, for example, will permit them to quantify the extent of problems caused by these fungi and develop specific strategies for their control.

Acknowledgements

The authors thank Dr. Ian Hall and Prof. Ursula Peintner for their manuscript presubmission review and for the help in the arrangement of the manuscript.

Literature Cited

Agerer R. 1987-2002. Colour Atlas of Ectomycorrhizae. Einhorn-Verlag, Schwäbisch Gmünd.

Agerer R. 1991. Characterization of ectomycorrhiza. In: Norris JR, Read DJ, Varma A. (eds.) Techniques for the study of mycorrhiza. Methods in microbiology, vol 23, pp. 25-73. Academic Press, London.

Altshul SF, Madden TL, Schaffer AA, Zhang JH, Zhang Z, Miller W, Lipman DJ. 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acid Research 25: 3389-3402.

Amicucci A, Potenza L, Guidi C, Rossi I, Bertini L, Zambonelli A, Stocchi V. 2002. Molecular techniques in the study of edible ectomycorrhizal mushrooms. In: Hall I, Wang Y, Danell E, Zambonelli A. (eds.) Edible Mycorrhizal Mushrooms and their Cultivation, Proceedings of the Second International Conference on Edible Mycorrhizal Mushrooms, 3-6 July 2001, Christchurch. New Zealand Institute for Crop and Food Research, CD-ROM.

Amicucci A, Rossi I, Potenza L, Zambonelli A, Agostini D, Palma F, Stocchi V. 1996. Identification of ectomycorrhizas from *Tuber* species by RFLP analysis of the ITS region. Biotechnology Letters 18: 821-826.

Bon M. 1997. Clé monographique du genre *Inocybe* (Fr.) Fr. (2ème partie: sous-genre *Inocybe* = *Inocybium* (Earle) Sing.). Documents Mycologiques 28: 1-77.

Brundrett M, Bougher N, Dell B, Grave T, Malajczuk N. 1996. Working with Mycorrhizas in Forestry and Agriculture. Australian Centre for International Agricultural Research, Monograph 32, Canberra.

Chevalier G, Frochot H. 1997. La truffe de Bourgogne (*Tuber aestivum* Chatin). Pétrarque, Levallois-Perret.

Dahlberg A. 2001. Community ecology of ectomycorrhizal fungi: an advancing interdisciplinary field. New Phytologist 150: 555-562.

De Miguel AM, Sáez R. 2005. Algunas micorrizas competidoras de plantaciones truferas. Publicationes de Biologia, Universidad de Navarra, Serie Botanica16: 1-18.

Deacon JW, Fleming LV. 1992 Interactions of ectomycorrhizal fungi. In: Allen MJ. (ed) Mycorrhizal Functioning an Integrative Plant-Fungal Process, pp. 249-300. Chapman and Hall, New York.

Donnini D, Bencivenga M. 1995. Micorrize inquinanti frequenti nelle piante tartufigene. Nota 2-Inquinanti in campo. Micologia Italiana 24: 185-207.

Franchi P, Gorreri L, Marchetti M, Monti G. 2001. Funghi di ambienti dunali. Grafiche 2000, Ponsacco.

Giomaro G, Sisti D, Zambonelli A, Amicucci A, Cecchini M, Comandini O, Stocchi V. 2002. Comparative study and molecular characterization of ectomycorrhizas in *Tilia americana* and *Quercus pubescens* with *Tuber brumale*. FEMS Microbiology Letters 216: 8-14.

Giomaro G, Zambonelli A, Sisti D, Cecchini M, Evangelista V, Stocchi V. 2000. Anatomical and morphological characterisation of mycorrhizas obtained *in vitro* using *Tilia platyphyllos* Scop. plantlets and five different strains of *Tuber borchii* Vittad. Mycorrhiza 10: 107-114.

Giovanetti G. 1983. Nella coltivazione il futuro del tartufo bianco. L'Informatore Agrario 39: 27463-27466.

Heim R. 1931. Le genre *Inocybe,* précédé d'une Introduction générale à l'Étude des Agarics Ochosporés. Encyclopédie Mycologique 1. Paul Lechevalier & Fils, Paris.

Horton TR, Bruns TD. 2001. The molecular revolution in ectomycorrhizal ecology: peeking into the black-box. Molecular Ecology 10: 1855-1871.

Izzo A, Agbowo J, Bruns TD. 2005. Detection of plot-level changes in ectomycorrhizal communities across years in an old-growth mixed-conifer forest. New Phytologist 166: 619-629.

Kuyper TW. 1986. A revision of the genus *Inocybe* in Europe. I. Subgenus *Inosperma* and the smooth-spored species of Subgenus *Inocybe.* Persoonia Suppl. 3: 1-247.

Molina R, Massicotte H, Trappe JM. 1992. Specificity phenomena in mycorrhizal symbioses: community-ecological consequences and practical implications. In: Allen MJ. (ed) Mycorrhizal Functioning an Integrative Plant-Fungal Process, pp. 357-423. Chapman and Hall, New York.

Moser M, Jülich W, Furrer-Ziogas C. 1994. Farbatlas der Basidiomyceten, III/29 *Inocybe splendens.* Gustav Fischer Verlag, Stuttgart.

Moser M, Jülich W, Furrer-Ziogas C. 1996. Farbatlas der Basidiomyceten, III/38 *Inocybe rufuloides.* Gustav Fischer Verlag, Stuttgart.

Pirazzi R. 2001. *Tuber magnatum* Pico: un fungo micorrizogeno tardivo. In: Actes du V^{e} Congrès International: Science et Culture de la Truffe, 4-6 mars 1999, Aix en Provence, pp. 233-236. Federation Française des Trufficulteurs, Paris.

Richard F, Millot S, Gardes M, Selosse MA. 2005. Diversity and specificity of ectomycorrhizal fungi retrieved from an old-growth Mediterranean forest dominated by *Quercus ilex*. New Phytologist 166: 1011-1023.

Royal Botanic Garden 1969. Flora of British Fungi. Colour identification chart. Her Majesty's Stationery Office, Edinburg.

Selosse MA. 2001. Avancées récentes dans l'étude des communautés et des populations ectomycorhiziennes. Lejeunia 165: 1-108.

Sisti D, Giomaro G, Cecchini M, Faccio A, Novero M, Bonfante P. 2003. Two genetically related strains of *T. borchii* produce *Tilia* mycorrhizas with different morphological traits. Mycorrhiza 13: 107-115.

Sourzat P. 2001. Evolutions technologiques et écologiques de la trufficulture en France et notamment dans le département du Lot. In: Actes du V^{e} Congrès International: Science et Culture de la Truffe, 4-6 mars 1999, Aix en Provence, pp. 419-424. Federation Française des Trufficulteurs, Paris.

Stangl J. 1989. Die Gattung *Inocybe* in Bayern. Hoppea 46. Denkschriften der Regensburgishen Botanischen Gesellschaft, Regensburg.

Trappe JM. 1987. Phylogenetic and ecologic aspects of mycotrophy in the angiosperms from an evolutionary standpoint. In: Safir GR. (ed) Ecophysiology of VA Mycorrhizal Plants, pp. 5-25. CRC Press, Boca Raton.

White TJ, Bruns T, Lee S, Taylor J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ. (eds.) PCR protocol: a guide to methods and applications, pp. 315-322. Academic Press, S. Diego.

Yamada A, Katsuya K. 2001. The disparity between the number of ectomycorrhizal fungi and those producing fruit bodies in a *Pinus densiflora* stand. Mycological Research 105: 957-965.

Zambonelli A, Iotti M, Zinoni F, Dallavalle E, Hall IR. 2005. Effect of mulching on *Tuber aestivum* ectomycorrhizas in an experimental truffière. New Zealand Journal of Crop and Horticultural Science 33: 65-73.

Zambonelli A, Iotti M, Giomaro G, Hall IR, Stocchi V. 2002. *T. borchii* cultivation: an interesting perspective. In: Hall I, Wang Y, Danell E, Zambonelli A. (eds.) Edible Mycorrhizal Mushrooms and their Cultivation, Proceedings of the Second International Conference on Edible Mycorrhizal Mushrooms, 3-6 July 2001, Christchurch. New Zealand Institute for Crop and Food Research, CD-ROM.

Zambonelli A, Iotti M, Rossi I, Hall IR. 2000. Interaction between *Tuber borchii* and other ectomycorrhizal fungi in a field plantation. Mycological Research 104: 698-702.

MYCOTAXON

Volume 94, pp. 85–88 October–December 2005

Russula in Himalaya 1: A new species of subgenus *Amoenula*

K. Das[1*], S.L. Miller[2], J.R. Sharma[3],
P. Sharma[4], & R.P. Bhatt[4]

daskanad@yahoo.co.in
[1] *Mycology and Plant Pathology Group, Agharkar Research Institute*
G.G. Agarkar Road, Pune 411 004, India

[2]*Botany Department, University of Wyoming*
Laramie, Wyoming 82071 USA

[3]*Botanical Survey of India, 192, Kaulagarh Road*
Dehradun 248195, India

[4]*Department of Botany, H.N. Bahuguna Garhwal University*
Srinagar (Garhwal) 246174, India

Abstract—*Russula mukteshwarica,* a species closely related to *R. violeipes,* is proposed here as new to science. Macro- and micromorphological characters of this species are described and illustrated in detail.

Key words—macrofungi, *Russulaceae*, taxonomy, phylogeny, India

Introduction

Extensive investigation on Himalayan *Russulaceae* has been carried out by the authors (Das & Sharma 2003, Das et al. 2003, Das & Sharma 2004, Das et al. 2004, Das & Sharma 2005, Das et al. 2005) for the last eight years. During macrofungal surveys of Kumaon and Garhwal Himalaya, the authors came across a species of *Russula* which after thorough macro- and microscopic studies followed by molecular analysis appeared to be an undescribed taxon and is proposed here as *Russula mukteshwarica.*

Materials and Methods

Macroscopic characters were noted from fresh material. Microscopic characterization was done from dried material by mounting free hand basidiome sections in 5% KOH, Melzer's reagent, Congo red, Lactophenol-cotton blue and Carbol Fuchsin. Colour terms follow Kelly & Judd (1955). Microscopic line drawings were made with the aid of a camera lucida at original magnification of 1500x for basidiospores, 1000x for other microstructures. Density of lamellae (L/cm) was measured at the margin of the pileus excluding lamellulae. Spore print colour follows Romagnesi (1967). Basidiospores length excludes the length of ornamentation. Basidium length excludes the length of sterigmata.

Quotient (Q = L/W) was calculated considering the mean value of length and width of 25 basidiospores. Herbarium names used follow Holmgren et al. (1990). Materials and methods for rDNA sequencing followed those of Miller & Henkel (2004).

Description of the species

Russula mukteshwarica K. Das, S.L. Mill., J.R. Sharma & R.P. Bhatt **sp. nov.**

Figure 1

> *Etymology*: From Mukteshwar, referring to the type locality.
>
> *Pileus 65–130 mm diam., planoconvexus, leviter depressus in centro, purpureus ad violaceus. Lamellae adnatae, confertae, luteae. Stipes 45–87 x 14–27 mm, cylindricus, purpureus. Sporae in cumulo albae, 7.6–9.3 x 7.3–8.2 µm, subglobosae vel globosae, amyloideae, verrucosae et incomplete reticulatae. Pleurocystidia 80–110 x 11–17 µm, fusiformia. Cheilocystidia 70–100 x 11–16 µm. Pileipellis bistrata.* ***Typus:*** *INDIA, Uttaranchal, Nainital, Mukteshwar, August 2003, leg. K. Das, KD2120 (HOLOTYPUS, BSD; ISOTYPUS, TUR-A)*

Pileus 65–130 mm diam., planoconvex, becoming umbilicate with depressed center at maturity; pileipellis dry, viscid when moist, pruinose to subvelvety, dark purple, deep to very deep purple or deep to dark violet, light to brilliant or very greenish yellow at the center; margin slightly decurved, almost plain at maturity, irregularly lobed, splitted, peeling up to 1/4th of the radius. **Lamellae** broadly adnate to adnexed, close (ca 7–9 per cm), forked from the base, brittle, cream to pale yellow or light greenish yellow; lamellulae present; edges even. **Stipe** 45–87 x 14–27 mm, cylindric to subclavate, dry, pruinose, light reddish purple at the top, gradually with white areas downwards but always with a white rim between the juncture of lamellae and stipe, pale greenish yellow (lemon yellow) at the base. **Context** stuffed, cream, changing light brown with phenol. **Taste** mild. **Spore print** white (Ia).

Basidiospores 7.6–9.3 x 7.3–8.2 µm, subglobose to globose (Q = 1–1.15, av. 1.05–1.10); ornamentation amyloid, composed of warts (=0.75 µm long) and ridges forming incomplete reticulum. **Basidia** 30–45 x 7–9 µm, subclavate to clavate, 4-spored; sterigmata =6.5–7 µm. **Pleurocystidia** 80–110 x 11–17 µm, abundant, emergent up to 40 µm, ventricose, subfusiform to fusiform, thick walled, content dense; wall up to 1.3 µm thick. Lamellae edge fertile, composed of basidia and cystidia. **Cheilocystidia** 70–100 x 11–16 µm, thick walled, same as pleurocystidia. **Subhymenium** layer narrow, up to 12 µm thick, cellular. **Pileipellis** two layered; upper layer composed of suberect to erect subulate, septate hyphae, 5–11 µm broad, pileocystidia absent; subpellis cellular.

Ecology – *Russula mukteshwarica* grows in close association with species of *Quercus, Rhododendron* and *Myrica* in moist deciduous to mixed subtropical to temperate (1800–2200 m) forests.

> **OTHER SPECIMENS EXAMINED**: **INDIA, Uttaranchal**: NAINITAL, Mukteshwar, August 2003, leg. K. Das, KD2127; PITHORAGARH, Dafia Dhura, September 2001, leg. K. Das & J.R. Sharma, KD4082; PAURI, Nagdev forest on path to Snake Temple, June 2003, leg. P. Sharma & S.L. Miller, PR310; *ibid.*, June 2003, leg. S.L. Miller & P. Sharma, SLM 03-04, SLM 03-07; *ibid.*, Khirsu, June 2003, leg. S.L. Miller & P. Sharma, SLM 03-22.

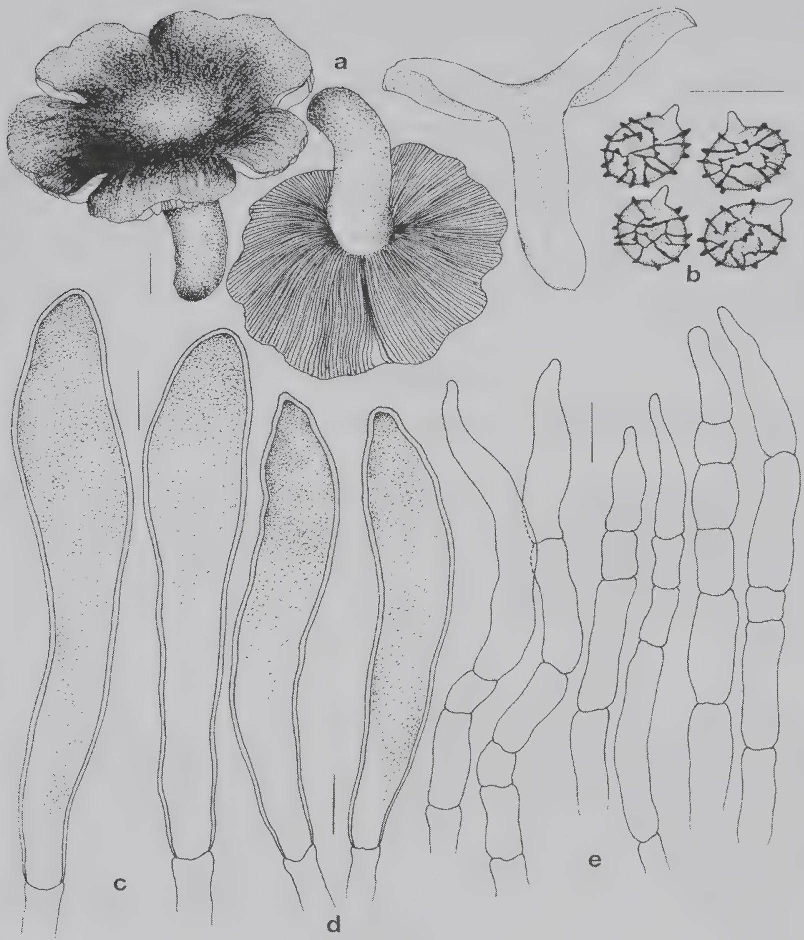


Fig. 1. *Russula mukteshwarica* (from holotype). a. Basidiomes. b. Basidiospores. c. Pleurocystidia. d. Cheilocystidia. e. Cross-section of pileipellis. Bars: a = 10 mm; b-e = 10 µm.

Comments – Typically subulate, up to 70 µm long hyphal ends of pileipellis and absence of dermatocystidia undoubtedly place the taxon in the subgenus *Amoenula* Sarnari. This species resembles *Russula amoenicolor* Romagn., *R. violeipes* Quél. and *R. amoena* Quél. from Europe. However, all these three species have a different spore print colour which varies from IIa–IIc (Romagnesi 1996), or from IIa–IIIa (Sarnari 1998). Moreover, the red to purple colour of stipe base in *R. amoenicolor*; presence of distinctly globose lower cells of the pilear hyphae in *R. violeipes* and the comparatively narrower pleurocystidia and reddish stipe base in *R. amoena* further separate these three species from *R. mukteshwarica*. Molecular analysis gathered from rDNA sequencing of the ITS gene region (not shown) has also confirmed that the new species belongs to the subgenus *Amoenula* and is closely related to *R. violeipes*.

Acknowledgements

We wish to express our gratitude to Dr. M. Sanjappa, Director and Dr. D.K. Singh, Joint Director, Botanical Survey of India, Kolkata and Dr. V.S. Rao, Director, Agharkar Research Institute, Pune for providing facilities during the present study. We are thankful to Mr. Jukka Vauras, Åbo akademi University (Turku, Finland) and Dr. L.L. Norvell (USA) for critically reviewing the manuscript and checking the Latin diagnosis. This research was also partially supported by funding to S.L. Miller from the National Science Foundation (DEB-0315607), USDA (2003-01542) and EPSCoR (04-47681). We gratefully acknowledge Terry McClean from the Nucleic Acid Exploration Facility at the University of Wyoming for sequencing these specimens.

Literature Cited

Das K, Sharma JR. 2003. New and interesting species of *Lactarius* from India. Mycotaxon 88: 377–385.

Das K, Sharma JR. 2003. New records of *Russula* from Kumaon Himalaya. Indian Journal of Forestry 26 (3): 320–326.

Das K, Sharma JR. 2004. *Lactarius* in Kumaon Himalaya 2: New and interesting species of subgenus *Plinthogali*. Mycotaxon 89 (2): 289–296.

Das K, Sharma JR. 2005. New records of *Lactarius* from India. Annals of Forestry 13 (1): 1–8.

Das K, Sharma JR, Verbeken A. 2003. New species of *Lactarius* from Kumaon Himalaya, India. Mycotaxon 88: 333–342.

Das K, Sharma JR, Montoya L. 2004. *Lactarius* (*Russulaceae*) in Kumaon Himalaya. 1. New species of subgenus *Russularia*. Fungal Diversity 16: 23–33.

Das K, Sharma JR, Basso MT, Bhatt, RP. 2005. *Lactarius* (*Russulaceae*) in Kumaon Himalaya 4: A new species of subgenus *Piperites*. Mycotaxon 91: 1–7.

Holmgren PK, Holmgren NH, Barnett LC. 1990. Index Herbariorum. Part 1: Herbaria of the world, 86th ed. Bronx: New York Botanical Garden.

Kelly KL, Judd DB. 1955. The ISCC-NBS Method of Designating Colors and a Dictionary of Colour Names. ISCC-NBS Color-Name Charts Illustrated with Centroid Colors. National Bureau of Standards Circular 553. U.S. Government Printing Office, Washington, DC.

Miller SL, Henkel TW. 2004. Biology and molecular ecology of subiculate *Lactarius* species from Guyana. In C.L. Cripps, ed., Fungi in forest ecosystems – systematics, diversity and ecology. New York Botanic Garden, vol. 89, pp. 297–313.

Romagnesi H. 1996. Les russues d'Europe et d'Afrique du nord. Bordas, Paris.

Sarnari M. 1998. Monografia illustrata del genere *Russula* in Europa. Tomo Primo. Italia. 799 pp.

MYCOTAXON

Volume 94, pp. 89–92 October–December 2005

Streptopodium passiflorae comb. nov. on *Passiflora rubra*

J. R. Liberato [1] & R. W. Barreto [2]

jose.liberato@dpi.qld.gov.au

[1] Department of Primary Industries & Fisheries, Plant Pathology Herbarium
80 Meiers Rd, Indooroopilly, Qld 4068, Australia

[2] Universidade Federal de Viçosa, Departamento de Fitopatologia
Viçosa, MG, 36571-000, Brazil

Abstract — Reexamination of *Ovulariopsis passiflorae*, the causal agent of a powdery mildew on *Passiflora rubra*, revealed that the fungus has dimorphic conidia and conidiophores formed from the external mycelium, a combination of features typical of *Streptopodium*. An emended description of this species is provided and the new combination *Streptopodium passiflorae* is proposed.

Key words — *Erysiphaceae, Phyllactinieae*

Introduction

Ovulariopsis passiflorae Syd. (*Erysiphaceae*) was described on *Passiflora rubra* L. based on a specimen collected in Venezuela by Sydow (1930). Its published description suggested that its maintenance in *Ovulariopsis* Pat. & Har. under the present concept for the genera in the *Phyllactinieae* (Braun et al. 2002) might be inadequate. A re-examination of the type specimen was made, confirming this suspicion. This paper reports the results of this new study of the type material and the nomenclatural change that resulted from it.

Material and Methods

In order to elucidate whether the conidiophores were produced from external or internal mycelium, a critical feature for separating genera in the *Phyllactinieae*, a whole leaf clearing and staining technique was used. Leaf pieces were immersed in solution of 50 g chloral hydrate in 20 mL of distilled water and left in stoppered glass vials at room temperature, for 24 h. The leaf pieces were then mounted on microscope slides in 85% lactic acid with aniline blue 1 g/L (Liberato et al. 2005). Only turgid and mature conidia (those unattached to conidiophores) were measured.

Results

Dimorphic conidia were found, feature that excludes the possibility of this fungus belonging to *Ovulariopsis*. The leaf clearing and staining technique enabled the visualization of superficial hyphae entering the leaves through stomata, an indication that the fungus has hemiendophytic mycelium (partly external and partly internal). With a single exception [species belonging to Cystotheca (Braun 1987)], this feature is exclusive to the tribe *Phyllactinieae* (Braun et al 2002). This technique also enabled the visualization of conidiophores arising from external mycelium. This combination of characters clearly indicates that the fungus belongs in the genus *Streptopodium* R.Y. Zheng & G.Q. Chen emend. (Liberato et al. 2004).

Taxonomic Description

Streptopodium passiflorae (Syd.) Liberato & R.W. Barreto **comb. nov. (emended)**

Figs. 1-8

≡ *Ovulariopsis passiflorae* Syd. Ann. mycol. 28(1-2): 199, 1930.

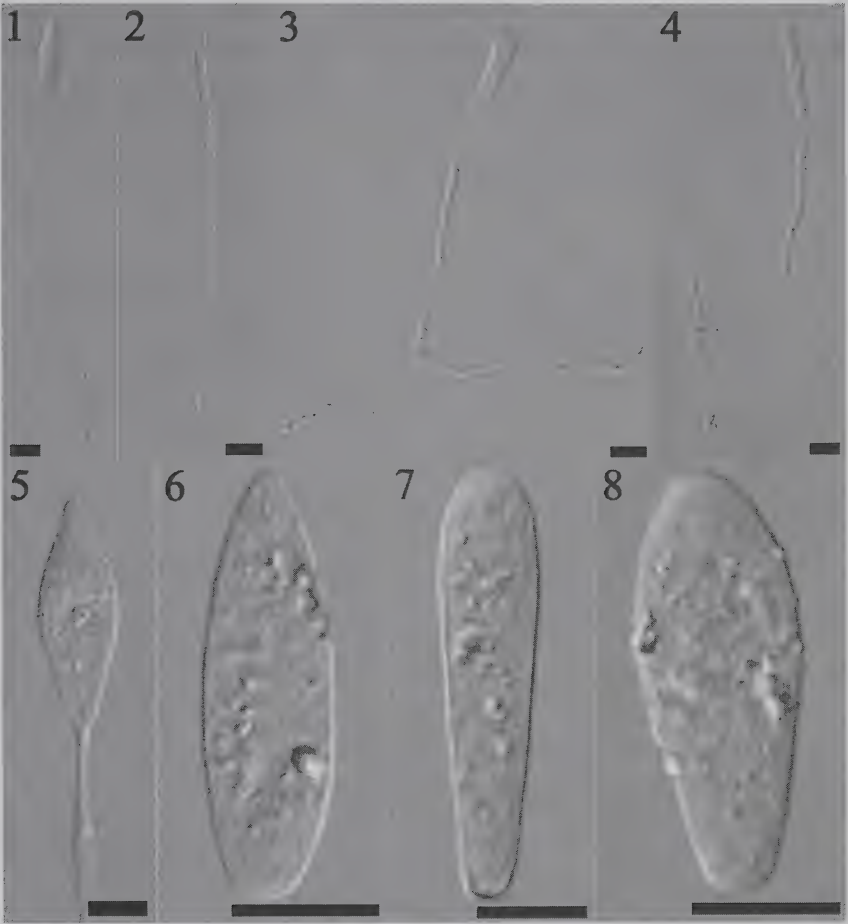
On living leaves. *Mycelium* hypophyllous. Superficial *hyphae* branched, septate, hyaline, flexuous, 4–6 µm wide, mycelial appressoria indistinct. *Conidiophores* produced from the external mycelium, cylindrical, hyaline, smooth, unbranched, septate, up to 264 x 5-7 µm, foot-cells mostly straight or somewhat slightly sinuous, very long, followed by 1-2 shorter cells. Conidia single, dimorphic: primary conidia lanceolate, apically pointed, 54–84 x 14–28 µm, l/w ratio 1.9–5.1; secondary conidia cylindrical to clavate with apically rounded, basally subtruncate ends, 50–76 x 16–26 µm, l/w ratio 1.9–4.8, aseptate, hyaline, smooth. One germ tube per conidium, up to 2 × the length of the conidium, with indistinct appressoria. Primary conidium with germ tube at base or at apex, secondary conidium with germ tube arising from the conidial shoulder. Teleomorph: not found.

SPECIMENS EXAMINED – VENEZUELA, La Victoria, Aragua, on *Passiflora rubra* L., 31 Jan 1928, H. Sydow (**HOLOTYPE**: BPI 414143; **ISOTYPE**: K(m) 131785).

Discussion

There are few reports of powdery mildew on *Passiflora*. *Oidium passiflorae* Politis was described from Greece (Politis 1938) and also reported in Germany (Braun 1998) and Australia (Liberato 2006). Anamorphic *Leveillula taurica* (Lév.) G. Arnaud was reported in Australia (Liberato 2006). Amano (1986) listed *Passiflora* spp. as hosts of these three species in some countries, although this author did not provide the original references from such records. The status of the two South African specimens, listed as *Ovulariopsis passiflorae* (Doidge 1950), and of *Ovulariopsis* sp. reported in Colombia (Tamayo & Pardo Cardona 2000) remain to be clarified. Misplacement of members of *Streptopodium* in *Ovulariopsis* appears to have occurred with some frequency in the past. For instance the powdery mildew of *Tabebuia serratifolia* (Vahl) G. Nicholson, originally identified by Ferreira (1989) as *Ovulariopsis* sp. was recently recollected and described as the new species *Streptopodium tabebuiae* Liberato & R.W. Barreto (Liberato & Barreto, 2005). This work represents part of an ongoing study aimed at elucidating the status of some dubious *Ovulariopsis.*

Now, there are five species included in *Streptopodium*: *S. bonariense* (Speg.) R.Y. Zheng & G.Q. Chen (teleom.: *Pleochaeta polychaeta* (Berk. & M.A. Curtis) Kimbr. & Korf) (Zheng & Chen 1978), *S. caricae* Liberato & R.W. Barreto (Liberato et al. 2004), *S. diospyri* G.J.M. Gorter (Gorter 1988), *S. passiflorae* and *S. tabebuiae* (Liberato &



Figs 1-8. *Streptopodium passiflorae* (from isotype) (bar = 20 μm). **Figs 1-4.** Conidiophores. **Figs 5-6.** Primary conidia. **Figs 7-8.** Secondary conidia.

Barreto 2005). Moreover, three other species of *Pleochaeta* Sacc. & Speg. emend. have unnamed anamorphs belonging in *Streptopodium*: *P. indica* N. Ahmad, A.K. Sarbhoy & Kamal (Ahmad et al. 1995), *P. prosopidis* (Speg.) U. Braun (Braun 1987) and *P. shiraiana* (Henn.) Kimbr. & Korf (Zheng & Chen 1978, Gorter & Eicker 1983).

Acknowledgements

The author acknowledges the herbaria BPI and K(m) for specimen loans and thank Dr Uwe Braun (Martin-Luther-Universität, Germany), Dr Hyeon-Dong Shin (Korea University) and Dr M. Havrylenko (Universidad Nacional del Comahue, Argentina), who kindly reviewed the manuscript. J. R. Liberato acknowledges financial support from the Brazilian Fundação Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).

Literature Cited

Ahmad N, Sarbhoy AK, Kamal. 1995. New powdery mildews from India. Mycological Research 99: 374-376.

Amano K. 1986. Host range and geographical distribution of the powdery mildew fungi. Japan Scientific Societies Press, Tokyo.

Braun U. 1987. A monograph of the *Erysiphales* (powdery mildews). Beih. Nova Hedwigia 89: 1-700.

Braun U. 1998. Neufunde Echter Mehltaupilze (*Erysiphales*) aus der BR Deutschland. Schlechtendalia 1: 31-40.

Braun U, Cook RTA, Inman AJ, Shin HD. 2002. The taxonomy of the powdery mildew fungi. Pp. 13-55 in Bélanger RR, Bushnell WR, Dik AJ, Carver TLW (Eds.) The powdery mildews: a comprehensive treatise. APS Press, St. Paul.

Doidge EM. 1950. The South African fungi and lichens to the end of 1945. Bothalia 5: 1-1094.

Gorter GJMA. 1988. Die Suid-Afrikaanse *Erysiphaceae* (meeldouswamme). Annale Universiteit Stellenbosch, reeks A3, 3: 1-64.

Gorter GJMA, Eicker A. 1983. *Uncinula polychaeta, Pleochaeta* and *Streptopodium* in South Africa. Transactions of the British Mycological Society 81: 398-401.

Ferreira FA. 1989. Patologia florestal. Principais doenças florestais no Brasil. Sociedade de Investigações Florestais, Viçosa.

Liberato JR. 2006. Powdery mildew on *Passiflora* in Australia. Australasian Plant Pathology 35: 73–75.

Liberato JR, Barreto RW. 2005. Additions to the Brazilian *Erysiphaceae*: *Ovulariopsis durantae* sp. nov. and *Streptopodium tabebuiae* sp. nov. Fungal Diversity 18: 95-106.

Liberato JR, Barreto RW, Louro RP. 2004. *Streptopodium caricae* sp. nov, with a discussion on powdery mildew of papaya and an emended description of the genus *Streptopodium* and of *Oidium caricae*. Mycological Research, 108: 1185-1194.

Liberato JR, Barreto RW, Shivas RG. 2005. Leaf-clearing and staining techniques for the observation of conidiophores in the *Phyllactinioideae* (*Erysiphaceae*). Australasian Plant Pathology 34: 401-404.

Politis J. 1938. Contribution à l'étude des champignons des Îles Ioniennes. Praktika tes Akadémias Athenon, 13: 602-610.

Sydow H. 1930. Fungi venezuelani. Annales Mycologici 28: 29-224.

Tamayo MP, Pardo Cardona VM (2000). Primeros registros y observaciones sobre mildeos polvosos y blancos en granadilla (*Passiflora mollissima* (HBK) Bailey), curuba (*Passiflora ligularis* Juss.) y otras pasifloraceas en Colombia. ASCOLFI Informa 26 (5): 40-42.

Zheng RY, Chen GQ. 1978. Taxonomic studies on the genus *Pleochaeta* of China. II. The imperfect state of *Pleochaeta*: *Streptopodium* gen. nov. Acta Microbiologica Sinica 18: 181-188

MYCOTAXON

Volume 94, pp. 93–102 October–December 2005

Type revision of three *Termitomyces* species from India

B. -H. Tang[1,3] T. -Z. Wei[1] & Y. -J. Yao[1,2*]

yaoyj@sun.im.ac.cn
[1]Systematic Mycology and Lichenology Laboratory
Institute of Microbiology, Chinese Academy of Science
Beijing 100080, China

[2]Mycology Section, Royal Botanic Gardens, Kew
Richmond, Surrey TW9 3AB, UK

[3]Graduate School of Chinese Academy of Sciences
Beijing 100080, China

Abstract—Taxonomic status of three *Termitomyces* species described from India, viz. *T. longiradicatus, T. quilonensis* and *T. poonensis,* is discussed in this paper based on examination of the type material. *Termitomyces longiradicatus* is proved to be a synonym of *T. heimii,* and *T. quilonensis* and *T. poonensis* are conspecific with *T. eurhizus.*

Key words—*Tricholomataceae*, termite symbionts, nomenclature, taxonomy

Introduction

Termitomyces R. Heim is a paleotropical genus of agarics obligately symbiotic with termites belonging to the subfamily *Macrotermitinae* (*Isoptera*). The *Termitomyces* species are usually characterised by the termite association, pinkish spore print, prominent perforatorium on the pileus and the subterranean pseudorhiza connected to the comb in the termite nest. *Termitomyces* has been proved to be a monophyletic clade in *Agaricales* based on molecular phylogenetic analyses (Moncalvo et al. 2000, Aanen et al. 2002, Rouland-Lefevre et al. 2002, Frøslev et al. 2003).

Termitomyces was established with ten species and six forms in 1942 (Heim 1942a), and more species and combinations were subsequently proposed by Heim (1942b, 1948, 1951, 1952, 1958) and by Otieno (1964, 1968). The genus was monographed by Heim (1977) with 28 taxa, including 20 species and eight forms from Africa and Asia. In the following decades, many new taxa were introduced, e.g. four from China (He 1985, Zhang and Ruan 1986, Wei et al. 2004), seven from India (Natarajan 1975, 1977, 1979, Sathe & Daniel 1980, Sathe & Deshpande 1980, Dhancholia et al. 1991), three from South Africa (Reid 1975, Van der Westhuizen & Eicker 1990), one from Zambia (Pegler & Piearce, 1980) and another from Tanzania (Saarimäki et al. 1994), eight from Cameroon (Mossebo et al. 2002) and five from South America (Gómez 1995; this report requires confirmation because South America is beyond the distribution

---

[*] Author for correspondence

range of *Macrotermitinae*). In total, 68 taxa have been published in *Termitomyces*, with 81 names including combinations and autonames. The genus is currently placed in *Tricholomataceae* R. Heim ex Pouzar (Kirk et al. 2001).

The eight new taxa, including seven species and one form described from India are *T. albidolaevis* Dhanch. et al., *T. heimii, T. indicus* Natarajan, *T. longiradicatus, T. quilonensis, T. poonensis, T. radicatus* Natarajan and *T. microcarpus* f. *santalensis* R. Heim. Among these names, *T. albidolaevis* was invalidly published according to Art. 37.5 of International Code of Botanical Nomenclature (Greuter et al. 2000) and *T. indicus* was considered to represent *T. microcarpus* f. *santalensis* (Pegler & Vanhaecke, 1994). Many species of *Termitomyces* reported in India, e.g. *T. clypeatus* R. Heim (Purkayastha 1985), *T. eurhizus* (Purkayastha 1985), *T. heimii* (Natarajan 1979), *T. microcarpus* (Berk. & Broome) R. Heim (Natarajan 1983), *T. radicatus* (Natarajan 1977) and *T. striatus* (Beeli) R. Heim (Purkayastha 1985), are also reported from China (Wei & Yao 2003).

In an ongoing project of *Termitomyces* species in China and worldwide, collections from India, including the type material of *T. longiradicatus, T. quilonensis* and *T. poonensis,* were kindly made available for study by the curator of the Herbarium of Mycology and Plant Pathology Department, Agharkar Research Institute, Pune, India (AMH). Examination of those specimens, compared with collections preserved in Mycological Herbarium, Academia Sinica (HMAS), Herbarium of Cryptogams, Kunming Institute of Botany, Academia Sinica (HKAS) and Herbarium, Royal Botanic Gardens, Kew (K), reveals that *T. longiradicatus, T. quilonensis* and *T. poonensis* are later synonyms of other species of *Termitomyces.* The results of this study are presented in this paper.

Materials and Methods

Dried specimens from the herbaria listed above were examined both macroscopically and microscopically. The dried specimens were photographed and dimensions measured. Most of the type specimens have been damaged, especially that of *T. longiradicatus.* The following description is based on the examination of the herbarium material. For microscopic studies, free-hand sections of dried basidiomata, including lamellae and cutis, were prepared using a razor-blade and mounted in a 5% KOH solution. Size ranges of basidiospores, basidia, lamella hyphae and cutis were measured using an ocular micrometer. At least 30 basidiospores and 20 basidia of each mature specimen were measured except otherwise specified. The microscopic characters were drawn with the aid of a camera lucida.

Taxonomy

Termitomyces longiradicatus Sathe & J. T. Daniel [as '*longiradicata*'] in MACS Monograph No. 1. *Agaricales* (Mushrooms) of South West India (Pune): 102 (1981) [as 1980].

Figs 1 & 2

Pileus 1.2–5.0 cm diam., campanulate or convex when young and then becoming plano-convex with an umbonate perforatorium; surface pale brown at the center, greyish white elsewhere. *Lamellae* cream, 3.0–4.0 mm broad, sinuate, crowded with lamellulae. *Stipe* 2.0 cm or longer, 0.5–0.8 cm diam., central, cylindric; surface pale brown. *Pseudorhiza*

0.5–0.7 cm diam., over 6.0 cm long; surface pale brown; smooth. *Annulus* broad and thick, greyish white, double-ringed, persistent on the upper part of the stipe; consisting of narrow, parallelly arranged hyphae of 2.0–5.0 µm diam. *Context* fleshy, consisting of thin-walled, inflated hyphae, 3.0–20 µm diam. *Basidiospores* 7.0–8.5 × 4.0–6.0 µm, ellipsoid, inamyloid. *Basidia* 18.5–24 × 8.5–9.5 µm, tetrasporic, clavate. *Lamella-edge* heterogenous. *Cheilocystidia* 21–56 × 10.5–27 µm, pyriform to inflated pyriform, hyaline. *Pleurocystidia* 28–67 × 14.5–28 µm, numerous, thin-walled, pyriform, hyaline. *Hymenophoral trama* regular, with parallel, thin-walled, hyaline hyphae, 3.0–20 µm diam. *Subhymenial layer* narrow. *Pileipellis* an epicutis of radially parellel, repent hyphae, up to 5.5 µm diam.

> ***Specimen examined***: **India:** Kerala State, Peechi, solitary, associated with termite nest, sine date, sine collector, AMH 4522 (holotype).

The color of pileus, stipe and pseudorhiza was observed from the dried material of the holotype, but it was described in the protologue as 'white-cream with pale grey tint' for the pileus, 'white becoming creamish white' for the stipe and 'white' for the pseudorhiza, presumably from the fresh material. Pileus context and lamellae in the holotype are seriously damaged by insects. There were only a few lamellae, basidiospores, basidia and cystidia available for examination (Fig. 2), but the lamellae and the range of the size of microscopic characters were described according to the original description. The tissue of stipe and pseudorhiza is also badly damaged in the holotype and the interior structure cannot be determined.

Termitomyces longiradicatus was described as close to *T. heimii* but different from the latter in having much longer radicating stipe (25–32 cm when fresh, with pseudorhiza up to 29 cm below the ground) and well-formed cheilocystidia (Sathe & Daniel 1981). From the original illustration and the present observation, the length of the stipe described for the holotype by Sathe & Daniel (1981) must include the pseudorhiza. However, the long stipe (5–9 cm) and pseudorhiza (7–36 cm), together with numerous cheilocystidia in the hymenium, have been reported in *T. heimii* (Pegler & Vanhaecke 1994). The length of the pseudorhiza is determined by the depth of the termite comb and the cystidia may be absent or present in species of *Termitomyces.* These are, therefore, not constant taxonomic characters. Specimens with long pseudorhiza and numerous cheilocystidia are found in many other collections from Asia, e.g.

> **China:** Yunnan, Jinghong, bought on the local market, 3 Aug. 2003, T. -Z. Wei & Q. -B., Wang, W03-6, HMAS 77076; Mengla, Menglun, bought on the local market, 16 July 1990, J. -G. Shuai 1, HKAS 22668; the same locality, bought on the local market, 5 Aug. 2003, M. Li & T. -Z. Wei, W03-8, HMAS 77075. **India:** Andra Pradesh, Prakasam District, Kansukur, on termite hills, Nov. 1980, C. -P. Roo, K(M) 94757; Tamil Nadu, Madras, Chepauk, Madras University Campus, on termite nest, 8 Nov. 1977, K. Natarajan, KN129, K(M) 94755 (holotype of *T. heimii* Natarajan). **Malaysia:** Selangor, Serdang, University Patonian, symbiosis with Odontotermes sp., 9 July 1990, M. Vanhaecke T3, K(M) 16528. Pakistan: Daska, on termite nests, 6 Aug. 1980, Ahmad 27757, K(M) 94753; Islamabad, on termite nests, 6 Aug. 1977, Ahmad 27462, K(M) 94647.

All these collections share the same characters of *T. heimii*, as indicated by examination of the type of the species. The morphological characters described for *T. longiradicatus* by Sathe & Daniel (1981) and confirmed by the present study are within the range of variation of *T. heimii.*

Termitomyces heimii was originally described from Madras, Tamil Nadu, India (Natarajan 1979) and is widely reported from tropical Southeast Asia (Natarajan & Raman 1983, Purkayastha 1985, Pegler & Vanhaecke 1994, Turnbull & Watling 1999) and southern China (Wei & Yao 2003). It is recognized by its large, smooth, white (grey or brownish grey at the centre), subumbonate pileus and persistent annulus (Natarajan 1979, Pegler & Vanhaecke 1994). The large, white-cream (dark at the center), umbonate pileus and the thick, persistent, broad, double-ringed annulus (Sathe & Daniel 1981, and this study) make *T. longiradicatus* identical with *T. heimii*. Therefore, they are considered conspecific here and *T. longiradicatus* is a later synonym of *T. heimii* Natarajan in Mycologia 71: 853 (1979).

Most collections of *T. heimii* examined here were collected in July and August in China and Malaysia, while the collections from India, including the holotype, were made in November. There is another record of *T. heimii* from China, which was made in the late season of the year (Yunnan, Mengla, Menglun, bought on the local market, 12 Nov. 1989, Z. -L. Yang 984, HKAS 22118), but with the stipe enlarged at the position of annulus (Yang 1990). The late season in China and the enlarged stipe make the collection very interesting for further study to compare with other material of the species from India and other countries of Asia.

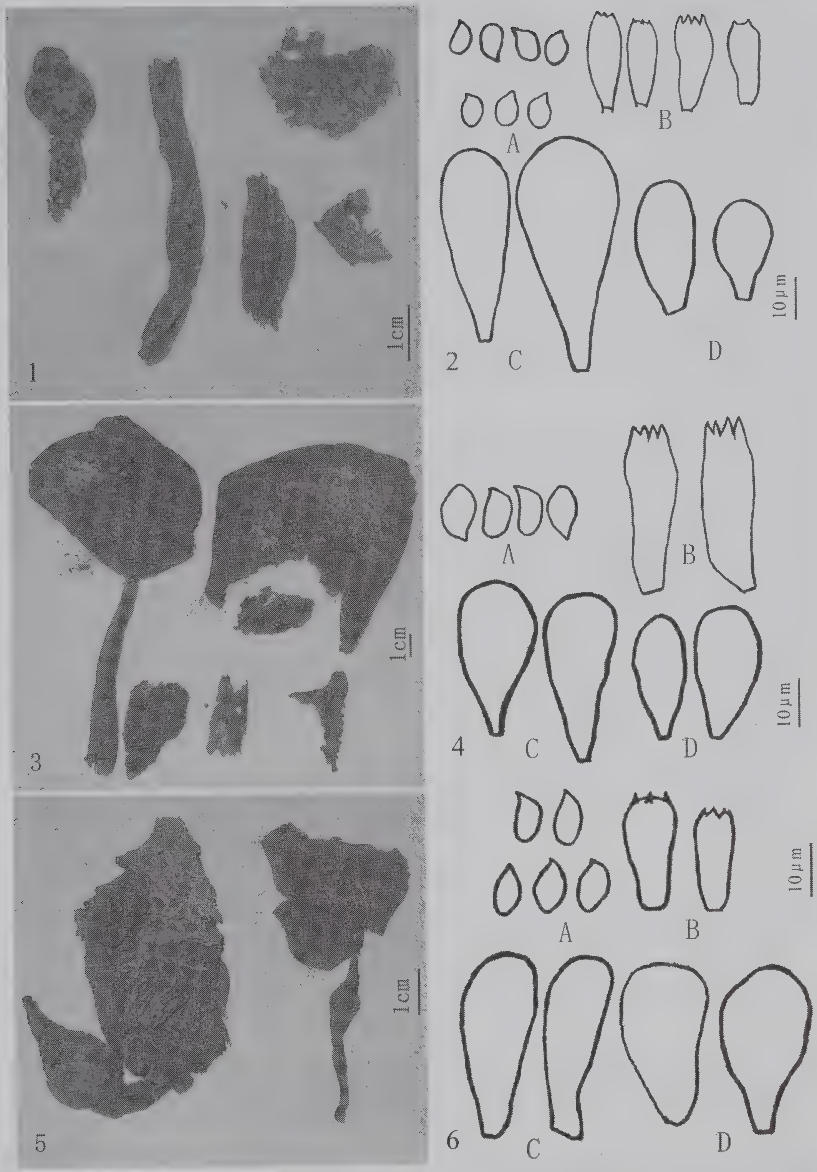
Termitomyces poonensis Sathe & S. D. Deshp. in MACS Monograph No. 1. *Agaricales* (Mushrooms) of South West India (Pune): 36 (1981) [as 1980]. **Figs 3 & 4**

Pileus 6.0–7.8 cm diam., plano-convex with a prominently rounded perforatorium, margin incurved with radially-arranged fine grey striae and spots; surface dark brown at the centre, brown or orange brown elsewhere, smooth. *Lamellae* free, up to 4.0 mm wide. *Stipe* over 9.5 cm long, 0.4–1.0 cm diam., central, cylindric at upper part, thickening downward up to 1.8 cm diam. at ground level; surface grey, becoming orange brown downwards, smooth and glabrous. *Pseudorhiza* over 3.5 cm long, tapering downward; surface black. *Annulus* absent. *Context* fleshy, of inflated, hyaline, thin-walled hyphae, 3.0–25 µm diam. *Basidiospores* 7.0–9.0 × 4.0–6.0 µm, ellipsoid, subhyaline and thin-walled. *Basidia* 15.0–20 × 7.0–12.0 µm. *Lamella-edge* heterogenous. *Cheilocystidia* 21–54 × 17.0–25 µm., obovoid to pyriform, hyaline, thin-walled *Pleurocystidia* 25–46 × 13.0–28 µm, pyriform, hyaline, thin-walled. *Hymenophoral trama* regular, 40–60 µm wide, of hyaline, thin-walled hyphae, 2.5–15.0 µm diam. *Subhymenial layer* 10.0–15.0 µm wide, of branched hyphae, 2.0–5.0 µm diam. *Pileipellis* an epicutis of dense, radially parellel, repent hyphae, 2.0–5.5 µm diam.

Specimen examined—**India:** Poona, in groups, on termite nests, sine date, sine collector, AMH 4479 (holotype).

The color of basidiomata was observed from the herbarium specimen of the holotype, whilst the color of the pileus was described as 'smoke gray when young, becoming platinum blonde with age', the stipe as 'white' and the pseudorhiza as 'black' in the protologue, possibly based on fresh material.

Sathe & Deshpande (1981) proposed this new species on account of its 'perforatorium prominent and darker than pileus, monomorphic cheilocystidia, conidial elements on pileus'. However, the glabrous, exannulate stipe and the black pseudorrhiza are reminiscent of *T. eurhizus*. *Termitomyces eurhizus* is a species with a wide distribution



Figs 1–6. Photographs and line drawings of *Termitomyces* spp. from India. Figs 1–2. *Termitomyces longiradicatus* (AMH 4522, holotype). Fig. 1. Habit. Fig. 2. A. Basidiospores; B. Basidia; C. Pleurocystidia; D. Cheilocystidia. Figs 3–4. *Termitomyces poonensis* (AMH 4479, holotype). Fig. 3. Habit. Fig. 4. A. Basidiospores; B. Basidia; C. Pleurocystidia; D. Cheilocystidia. Figs 5–6. *Termitomyces quilonensis* (AMH 4546, holotype). Fig. 5. Habit. Fig. 6. A. Basidiospores; B. Basidia; C. Pleurocystidia; D. Cheilocystidia.

(Saccardo 1887, Pegler & Rayner 1969, Pegler 1977, Samajpati 1981, Pegler & Piearce 1980, Piearce 1987, Wei & Yao 2003), and its pileus color and form are variable. The color varies from grayish brown to dark brown and often darker in the center than elsewhere. The perforatorium is obtusely conical to mucronate (Pegler & Vanhaecke 1994) and often prominent (Singer 1949, Purkayastha & Chandra 1975, Samajpati 1981, Pegler 1986). The cheilocystidia are obovoid to pyriform (Pegler 1977, 1986, Pegler & Vanhaecke 1994) or usually pyriform (Purkayastha & Chandra 1975, Purkayastha 1985). There have been quite a few synonyms for this species, e.g. *T. cartilagineus* (Berk.) R. Heim (Pegler & Rayner 1969, Pegler 1977) and *Agaricus sparsibarbis* Berk. & Broome (Pegler & Vanhaecke 1994). *Termitomyces albiceps* S. C. He, based on some collections from Guizhou, China, was also suspected to be a synonym of *T. eurhizus* (Pegler & Vanhaecke 1994). The monomorphic cheilocystidia (Sathe & Deshpande 1981) of *T. poonensis* might be due to limited material examined, as the cheilocystidia of *T. albiceps* were also described as pyriform (He 1985). The 'transversely septate' cheilocystidia in the original description of *T. poonensis* were not found in the holotype by the present authors. The conidial elements on pileus, mentioned in the protologue, are possibly accidentally produced and have not been reported elsewhere for *Termitomyces.* Species of *Termitomyces* can produce sporodochia bearing conidiophores in the fungus garden within termite nest and the conidia produced by the conidiogenous hyphae were often similar in size and form (Botha & Eicker 1991b). The conidial elements mentioned in the protologue of *T. poonensis* are not a stable character and have never been used to distinguish species in the genus. Judged from the morphology of basidiomata observed from the holotype, *T. poonensis* is here considered a synonym of *T. eurhizus* (Berk.) R. Heim in Arch. Mus. Hist. Nat. Paris, Sér. 6, 18: 140 (1942).

To confirm the above determination, the following collections of *T. eurhizus* were also examined for comparison.

> **India:** Bishnupur, Shillong, Meghalaya, 3 July 1984, R. -N. Verma, M107, K(M) 94642. **China:** Yunnan, Mengla, Menglun, Xishaungbanna Tropical Botanical Garden, 8 Aug. 2003, G. -R. Hu & T. -Z. Wei, W03-27, HMAS 88326; the same locality and date, G. -R. Hu & T. -Z. Wei, W03-21, HMAS 84723; the same locality, 4 Aug. 1988, Z. -L. Yang 262, HKAS 21785; Yunnan, Simao, bought on the local market, 10 Aug. 2003, T. -Z. Wei & Q. -B. Wang, W03-30, HMAS 85229; Sichuan, Chengdu, bought on the local market, 30 Aug. 2002, B. Wang 200252, HMAS 84529. **Malaysia:** Selangor, Serdang, Malesysen, Universitat Pertanian, 7 July 1990, M. Vanhaecke, T9, K(M) 16525. **Sri Lanka:** Kandy District, Peradeniya Botanic Garden, alt. 1600ft, 23 Oct. 1974, D. N. Pegler 2062, K(M) 94646.

Termitomyces quilonensis Sathe & J. T. Daniel [as '*quilonesis*'] in MACS Monograph No. 1 *Agaricales* (Mushrooms) of South West India (Pune): 103 (1981) [as 1980].

Figs 5 & 6

Pileus 3.3–6.5 cm diam, convex or up-turned, with an obtusely conical perforatorium, slightly inflexed at the margin; surface dark brown at the centre and becoming paler toward the margin. *Lamellae* free, up to 3.0 mm wide. *Stipe* 5.0 × 0.2–1.0 cm, cylindrical, enlarged at ground level and forming a globose bulb up to 1.6 cm diam. below ground; surface dark brown to almost black, smooth; solid. *Pseudorrhiza* over 3.0 cm long, tapering abruptly downward below the bulb, solid; surface black. *Annulus* absent. *Context* fleshy, of inflated, hyaline, thin-walled hyphae, 3.0–8.0 µm diam. *Basidiospores* 8.0–9.0 × 4.5–5.5 µm, ellipsoid, subhyaline and thin-walled. *Basidia* 17.5–30 × 6.0–8.5 µm, subhyaline, thin-walled, clavate, tetrasporic. *Lamella-edge* heterogenous. *Cheilocystidia* 25–41 × 12.0–27 µm, pyriform to inflated pyriform, hyaline, thin-walled. *Pleurocystidia* 28–52 × 14.5–28 µm, rare, broadly clavate or pyriform to inflated pyriform, hyaline. *Hymenophoral trama* regular, 50–60 µm wide, of hyaline, thin-walled hyphae, 2.5–15.0 µm diam. *Subhymenial layer* 10.0–15.0 µm wide, of branched hyphae, 2.0–5.0 µm diam. *Pileipellis* an epicutis of dense, radially parellel, repent hyphae, 2.0–5.5 µm diam.

> ***Specimens examined*:** **India:** Kerala State, Quilin, solitary, on termite nest, sine date, sine collector, AMH 4546 (holotype).

The color of pileus, stipe and pseudorhiza in the above description was taken from the dried material of the holotype. In the protologue, the pileus was described as 'gray towards margin, brownish gray towards center', the stipe as 'white' and the pseudorhiza as 'brownish black' possibly from fresh material.

According to Sathe & Daniel (1981), *T. quilonensis* differs from *T. poonensis* in the absence of conidia and in having bulbous base in the stipe. However, the stipe base of *T. poonensis* is also enlarged slightly in the holotype as observed by the present authors. As conidia are not a useful character to distinguish species in the genus (see above in the remark for *T. poonensis*), absence of conidia cannot make *T. quilonensis* a distinct species from *T. poonensis.* Further, as discussed above, *Termitomyces poonensis* is a synonym of *T. eurhizus.* In fact, *T. eurhizus* is a species variable in morphology, but can be easily recognized by its black-brown encrusted and cartilaginous pseudorhiza (Heim 1942, Pegler 1977, 1986, Pegler & Vanhaecke 1994). The stipe of *T. eurhizus* often expands at the base before attenuating into an elongate pseudorhiza (Pegler & Rayner 1969, Pegler 1977), often but not always with a bulbous base (Pegler 1986). The brownish gray pileus and the black pseudorhiza of *T. quilonensis* closely resemble *T. eurhizus,* and the microscopic characters are all within the range of those of *T. eurhizus.* Thus, *T. quilonensis* is considered as another synonym of *T. eurhizus.*

Discussion

The above three species of *Termitomyces* were proposed based on minor morphological characters, e.g. cystidia, conidial elements, and the length or form of stipe or pseudorhiza (Sathe & Deshpande 1981). Cystidia may be absent or present in *Termitomyces* species (Van Der Westhuizen & Eicker 1990), and they could be found as either monomorphic or polymorphic based on different materials examined (Pegler 1977, Pegler & Vanhaecke 1994). Conidia are asexual spores produced in termite combs or in cultures. The size and form of conidia are similar in cultures, except for *T. microcarpus* which lacks conidiophores and conidia (Botha & Eicker 1991a) and the presence of conidia on the pileus surface might be accidental (Botha & Eicker 1991a). As the form of basidiomata in *Termitomyces* depends very much on the symbiosis, especially the identity and behaviour of the host termites (Piearce 1987), the length of pseudorhiza can vary with the depth of the termitaria. The swollen stipe base is present in several species of the genus, such as *T. umkowaani* (Cooke & Massee) D. A. Reid, *T. bulborhizus* T. Z. Wei et al. and *T. sagittiformis* (Kalchbr. & Cooke) D. A. Reid. However, the bulbous base is not stable in some species as discussed above in *T. eurhizus.*

Among the eight new taxa of *Termitomyces* described from India, two are well recognized, i.e. *T. heimii* with a wide distribution in Asia (Pegler & Vanhaecke 1994, Wei & Yao 2003) and *T. microcarpus* f. *santalensis* in India (Pegler & Vanhaecke 1994). However, in addition to *T. indicus* (=*T. microcarpus* f. *santalensis*, see Pegler & Vanhaecke, 1994), three more have been proved to be synonyms of other species in the genus by the present study, i.e. *T. longiradicatus, T. quilonensis* and *T. poonensis.* For the remaining two, *T. albidolaevis* and *T. radicatus,* they also need further examination. *Termitomyces albidolaevis* was proposed based on silvery white color of the pileus, considered close to *T. mammiformis* f. *albus* R. Heim but different in the absence of conical perforatorium and in having larger basidiospores (7.5–10.0 × 5.0–7.0 µm, Dhancholia et al., 1991), although the pileus was contradictorily described as 'umbonate' in the same publication. In fact, *T. albidolaevis* can be easily distinguished from *T. mammiformis* f. *albus* by the absence of the annulus and the size of basidiospores (6–8 × 3.5–4.5 µm in *T. mammiformis* f. *albus*, see Pegler 1977) according to the original description and illustration. *Termitomyces albidolaevis* is somewhat unique for its relatively large, white pileus (11–16 cm), the whitish pseudorhiza and large basidiospores. *Termitomyces citriophyllus* R. Heim is the most close species to *T. albidolaevis* in terms of the size of pileus (9–10 cm) and of basidiospores (9–11× 6.7–7.7 µm), but differs in the dark colored (ochreous gray) pileus, and much larger basidia (30–49 × 12–15 µm, compared with 17.0–27.5 × 5.0–7.5 µm in *T. albidolaevis*), although the surface color of pseudorhiza was never mentioned (Heim 1942a, 1977). Re-examination of the type material of *T. albidolaevis* may confirm its status. *Termitomyces radicatus* is distinguished from other species with small basidiomata, such as *T. microcarpus, T. medius* R. Heim & Grassé and *T. tylerianus* Otieno, by the dark colored spiniform perforatorium, a short pseudorhiza and the absence of pleurocystidia and cheilocystidia (Natarajan 1977). However, the pseudorhiza were found terminating at a sclerotized disk and scattered hymenial cystidia were detected in some material of *T. radicatus* from India and Pakistan (Pegler & Vanhaecke 1994). *Termitomyces radicatus* was also considered by Pegler & Vanhaecke (1994) to resemble *T. clypeatus* but much smaller and to differ from *T. microcarpus* mainly in the presence of a short pseudorhiza. The differences among *T. radicatus, T. medius* and *T. tylerianus* are also not very significant. *Termitomyces radicatus* was distinguished from the other two by the pseudorhiza termitnating at a sclerotized disc (Pegler & Vanhaecke 1994), but a disc-like structure in the base of the pseudorhiza was also observed in other species, such as *T. reticulatus* Van der Westh. & Eicker (Van der Westhuizen & Eicker 1990), although the latter is much larger in size. Observation of such a structure depends much upon the intactness of the collection. The terminal structure in the pseudorhiza of *Termitomyces* species may have been destroyed in most collections because the pseudorhiza is connected with the nest of termite. Detailed study of *Termitomyces* species with small basidiomata is required to reveal the relationships of those species. A molecular systematic study of these species is undergoing in this laboratory.

Acknowledgements

The authors are grateful to Drs Tobias Frøslev and Zhu-Liang Yang for serving as pre-submission reviewers and for their valuable comments and suggestions, to Drs Alaka Pande and Sanjay K. Singh of AMH for the loan of *Termitomyces* specimens. This project is supported by the National Hi-Tech

Research and Development Plan (2004AA227100) from the Ministry of Science and Technology, the National Science Fund for Distinguished Young Scholars (30025002) from National Natural Science Foundation of China, and the Key Research Direction of Innovation Programme (KSCX2-SW-101C) and the scheme of Introduction of Overseas Outstanding Talents, operated by the Chinese Academy of Sciences.

Literature Cited

Aanen DK, Eggleton P, Rouland-Lefèvre C, Frøslev TG, Rosendah S, Boomsma JJ. 2002. The evolution of fungus-growing termites and their mutualistic fungal symbionts. Proceedings of the National Academy of Sciences of the United States of America 99: 14887–14892.

Botha WJ, Eicker A. 1991a. Cultural studies on the genus *Termitomyces* in South Africa. I. Macro- and microscopic characters of basidiome context cultures. Mycological Research 95: 435–443.

Botha WJ, Eicker A. 1991b. Cultural studies on the genus *Termitomyces* in South Africa. II. Macro- and micromorphology of comb sporodochia. Mycological Research 95: 444–451.

Dhancholia S, Bhatt JC, Pant SK. 1991. Studies on some Himalayan Agarics. Acta Botanica Indica 19: 104–109.

Frøslev TG, Aanen DK, Læssøe T, Rosendahl S. 2003. Phylogenetic relationships of *Termitomyces* and related taxa. Mycological Research 107: 1277–1286.

Gómez LD. 1994 (1995). Una nueva especie neotropical de *Termitomyces* (*Agricales*: *Termitomycetaceae*). Revista de Biologia Tropical 42: 439–441.

Greuter W, Mcneill J, Barrie FR, Burdet H-M, Demoulin V, Filgueiras TS, Nicolson DH, Silva PC, Skog JE, Trehane P, Turland NJ, Hawksworth DL. 2000. International Code of Botanical Nomenclature (St Louis Code). Koeltz Scientific Books, Königstein, Germany.

He SC. 1985. Taxonomic studies of *Termitomyces* from Guizhou Province of China. Acta Mycologica Sinica. 4: 103–108.

Heim R. 1942a. Les champignons des termitières. Nouveax aspects d'un problème de biologie et de systématique générale. *Revue Scientifique* 80: 69–86.

Heim R. 1942b. Nouvelles Études sur Les Agarics Termitophiles d'Afrique Tropicale. Archives du Muséum National d'Histoire Naturelle, Séries. 6, 18: 1–60, pls 9–12.

Heim R. 1948. Nouvelles réussites culturales sur les *Termitomyces*. Comptes Rendus Hebdomadaires des Séances de l'Académie des Science 226: 1488–1491.

Heim R. 1951. Les *Termitomyces* du Congo Belge recueillis par Medame M. Goossens-Fontana. Bulletin du Jardin Botanique de l'État 21: 205–222.

Heim R. 1952. Les *Termitomyces* du Cameroun et du Congo francais. Neue Denksehriften der allgemeinen Schweizerschen Gesellschaft fur die gesammten Naturwissenschaften. 80: 1–29.

Heim R. 1958. *Termitomyces*. Flore Iconographique Champignons du Congo 7: 139-151, pl. 23–25.

Heim R. 1977. Termites et Champignons. Société Nouvelle Des Éditions Boubée, Paris.

Kirk PM, Cannon PF, David JC, Stalpers JA. 2001. Dictionary of the Fungi. 9th edn. CAB International, Wallingford, UK.

Moncalvo JM, Lutzoni FM, Rehner SA, Johnson J, Vilgalys R. 2000. Phylogenetic relationships of agaric fungi based on nuclear large subunit ribosomal DNA sequences. Systematic Biology 49: 278–305.

Mossebo DC, Amougou A, Atangana RE. 2002. Contribution a l'étude du genre *Termitomyces* (Basidiomycètes) au Cameroon: écologie et systematique. Bulletin de la Société Mycologique de France 118: 195–249.

Natarajan K. 1975. South Indian *Agaricales* I. *Termitomyces*. Kavaka 3: 63–66.

Natarajan K. 1977. A new species of *Termitomyces* from India. Current Science 46: 679–680.

Natarajan K.1979. South Indian *Agaricales* V: *Termitomyces heimii*. Mycologia 71: 853–855.

Natarajan K, Raman N. 1983. South Indian *Agaricales*. A list of species of Agarics and Boletes reported earlier from South India. New Delhi: International Books and Periodicals Supply Service: 169–182.

Otieno NC. 1964. Contributions to a knowledge of termite fungi in East Africa: the genus *Termitomyces* Heim. Proceedings of the East African Academy 2: 108–120.

Otieno NC. 1968. Further contributions to a knowledge of termite fungi in East Africa: the genus *Termitomyces* Heim. Sydowia 22: 160–165.

Pegler DN. 1977. A Preliminary Agaric Flora of East Africa. Kew Bulletin. Additional Series 6: 1–615.

Pegler DN. 1986. Agaric Flora of Sri Lanka. Kew Bulletin. Additional Series 12: 251–258.

Pegler DN, Piearce GD. 1980. The edible mushroom of Zambia. Kew Bulletin 35: 475–491.

Pegler DN, Rayner RW. 1969. A contribution to the Agaric flora of Kenya. Kew Bulletin 23: 347–412.

Pegler DN, Vanhaecke M. 1994. *Termitomyces* of Southeast Asia. Kew Bulletin 49: 717–736.

Piearce GD. 1987. The genus *Termitomyces* in Zambia. The Mycologist 21: 111–116.

Purkayastha RP. 1985. Manual of Indian Edible Mushrooms. New Delhi: India Today & Tomorrow's Printers and Publishers, pp.121–125.

Purkayastha RP, Chandra A. 1975. *Termitomyces eurhizus*, a new Indian edible mushroom. Transactions British Mycological Society. 64: 168–170.

Reid DA. 1975. Contributions from the Bolus Herbarium 7: Type studies of the larger *Basidiomycetes* described from Southern Africa. pp.1–255.

Rouland-Lefevre C, Diouf MN, Brauman A, Neyra M. 2002. Phylogenetic relationships in *Termitomyces* of ITS: a first approach to elucidate the evolutionary history of the symbiosis between fungus-growing termites and their fungi. Molecular Phylogenetics & Evolution 22: 423–429.

Saarimäki T, Härkönen M, Mwasumbi L. 1994. Tanzanian mushrooms and their use 3. *Termitomyces singidensis*, sp. nov. Karstenia 34: 13–20.

Saccardo PA. 1887. Sylloge Fungorm 5: pp.1–1146.

Samajpati N. 1981. Some tropical edible mushrooms from India. Mushroom Science 11: 45–54.

Sathe AV, Daniel J. 1981 [as 1980] . Maharashtra Association for the Cultivation of Science (MACS), Monograph No. 1: *Agricales* (Mushrooms) of south west India (Pune): 75–108.

Sathe AV, Deshpande S. 1981 [as 1980]. Maharashtra Association for the Cultivation of Science (MACS), Monograph No. 1; *Agaricales* (Mushrooms) of South West India (Pune): 9–42.

Singer R. 1945. New genera of fungi-II. Lloydia 8: 139–144.

Turnbull E, Watling R. 1999. Some records of *Termitomyces* from old world rainforests. Kew Bulletin 54: 731–738.

Van der Westhuizen GCA, Eicker A. 1990. Species of *Termitomyces* occurring in South Africa. Mycological Research 94: 923–937.

Wei T-Z, Yao Y-J. 2003. Literature review of *Termitomyces* species in China. Fungal Science 22: 39–54 (in Chinese).

Wei T-Z, Yao Y-J, Wang B, Pegler DN. 2004. *Termitomyces bulborhizus* sp. nov. from China, with a key to allied species. Mycological Research 108: 1458–1462.

Yang Z-L. 1990. A delicious tropical mushroom-*Termitomyces heimii* occurring in Yunnan, China. Edible Fungi of China 9: 28. (in Chinese).

Zhang Z-F, Ruan X-Y. 1986. A new species of *Termitomyces—Termitomyces macrocarpus* Zhang et Ruan sp. nov. Edible Fungi 5: 10–13. (in Chinese).

MYCOTAXON

Volume 94, pp. 103–110 October–December 2005

A review of the genus *Gyromitra* (Ascomycota, *Pezizales, Discinaceae*) in Mexico

Rosario Medel

medelr@ecologia.edu.mx
Instituto de Ecología, A.C. Unidad de Micología
Km 2.5 antigua carretera Xalapa-Coatepec No. 351
Congregación El Haya, 91070, Xalapa, Veracruz, México

Abstract—*Gyromitra* has three species in Mexico: *G. ambigua, G. esculenta* and *G. infula*, of which *G. ambigua* is recorded for the first time. The three species are widely distributed primarily in forest of *Abies* and *Pinus-Quercus* and rarely in cloud forests. Some considerations of the *G. esculenta* complex are presented and a key to the recognized species is provided.

Key words—taxonomy, species complex

Introduction

According to Kirk et al. (2001), the genus *Gyromitra* Fr. is composed of nearly fifteen species. Usually, it has been considered to be closely related to three genera: *Discina* (Fr.) Fr., *Neogyromitra* S. Imai and *Pseudorhizina* Jacz. These genera have a common excipular structure, four nuclei per spores and paraphyses that are very similar; differences in spore shape and ornamentation, ascoma shape and presence of a stipe are considered quantitative (Harmaja 1969a, 1973). The relationships among these and other genera are not clear (Harmaja 1969a). *Gyromitra* was placed first in the family Helvellaceae Dumort. (Saccardo1889; Seaver 1928; Raitviir 1965; Dennis 1978, Korf 1972, Kimbrough et al., 1990, Kimbrough 1991). However, O'Donnell et al. (1997) moved *Gyromitra* to the family Discinaceae Benedix emend. N.S. Weber, Trappe & O´Donnell, and they pointed out the necessity of doing phylogenetic studies within the genera of this family, including *Gyromitra* and argued, in agreement with Harmaja (1969a), that *Gyromitra* and *Discina* are congeneric (Eckblad 1968; Abbott and Currah 1997). Information on the position of the genus *Neogyromitra* (Harmaja 1969a) is still wanting. *Gyromitra* also needs a detailed survey to establish relationships among species. It may be composed of two (Harmaja, 1973) or more subgenera (Mcknight and Batra 1974; Abbott and Currah 1997).

Gyromitra sensu lato, that is including *Discina*, comprises species with inamyloid asci, elliptical, smooth ascospores, apothecia that are stipitate and bilobate or brain-like, as well as those species with subfusiform to fusiform ornamented ascospores, apothecia that are discoid, sessil or subsessil and medullary excipulum of textura intrincata. This is the generic concept presented by Harmaja (1969a, 1973) and Pfister (1980). In Mexico only the stipitate, bilobate or brain-like species have been found to date. *G. esculenta* is variable in spore form and I follow Harmaja´s (1969a) spore types in the discussion below to distinguish within this complex.

Gyromitra was recorded in Mexico for the first time by Herrera and Guzmán (1961), based on *G. infula* (as "*Helvella infula* Fr."), growing in coniferous forests in the State of Mexico and Federal District. There it has been known as an edible fungus, after being boiled and the water being discarded. Later *G. esculenta* was recorded from Nuevo León by Castillo et al. (1979). A third species *Gyromitra melaleuca* (Bres.) Donadini (as *Discina melaleuca* Bres.) was reported from Jalisco State by Guzmán-Dávalos et al. (2001).

Materials and methods

Eighty five specimens from the following herbaria were considered in this study: ENCB, FCF, FCME, FH, H, IBUG, ITCV, K and XAL. Acronyms follow Holmgren et al. (http://sciweb.nybg.org/science2/ih/searchih.html) with the addition of ITCV (Instituto Tecnológico de Ciudad Victoria) and FCF (Facultad de Ciencias Forestales, Universidad Autónoma de Nuevo León). Microscopic observations were made on sections mounted in 10% KOH, for measurements of hymenial elements, and Melzer reagent and cotton blue in lactophenol for observation of spore ornamentation and apiculi.

Results

Gyromitra in Mexico comprises three species: *G. ambigua*, *G. esculenta* and *G. infula. G. ambigua* has not previously been reported in Mexico. The report of *Gyromitra melaleuca* (as *Discina melaleuca,* Guzmán-Dávalos et al., 2001) is based on a misidentification. Study of reference material (*Tamayo & González* s.n., from Nevado de Colima mountain, in Jalisco, IBUG), showed a superficial similarity to *G. melaleuca*, i.e. apothecium discoid, with margin unevenly revoluted, and a short, nut-brown stipe; and spores 20-22 x 10-12 µm, with short spines and paraphyses 5-8 µm wide and clavate. Nevertheless, when mounted in Melzer reagent a positive reaction of the asci is observed indicating it is a member of the family *Pezizaceae*. It is worth note that the record of *G. melaleuca* from North America (Seaver 1928) is doubtful. Abbott and Currah (1997) suggest the collection from the United States represents a different species since they consider *G. melaleuca* to be restricted to Europe from where it was described.

Key to the recognized species of *Gyromitra* in Mexico

1a. Apothecium gyrose or brain-like or rarely bilobate, thick, vinaceous brown, reddish or violaceous, spores elliptical to fusoid 18-25 (-27) x (8-) 10-12.5 µm . . . . **G. esculenta**

1b. Apothecium saddle shape or rarely gyrose and narrow, brown orange or reddish-brown. 2

2a. Spores elliptical, walls without thickened poles, 17.6-25 x 7.2-10 µm . . . . **G. infula**

2b. Spores elliptical-fusoid, (23-) 25-33 x (8-)10-12 µm walls thickened at the poles forming apiculi up to 2.5 µm thick . . . . **G. ambigua**

Gyromitra ambigua (P. Karst.) Harmaja, Karstenia 9: 17. 1969. **Fig. 1-2**

≡ *Helvella ambigua* P. Karst., Meddelanden af Societas pro Fauna et Flora Fennica 5: 53. 1879.
= *Gyromitra infula* var. *apiculatispora* Raitv., Eesti NSV Teaduste Akadeemia Toimetised (Bioloogiline Seeria) 14 (3): 322. 1965 (fide Abbott and Currah, 1997)

The lobed or rarely brain-like, dark red-orange apothecia and fusoid spores, (23-) 25-33 x (8-) 10-12 μm, with thicker poles, as apiculi 2.5 μm high, are the diagnostic features. The ascoma of this species is very similar to *G. infula*, although the shape of some material studied resembles *G. esculenta*. We studied the holotype material and found the spores to measure 30-33 x 10-12 μm, with apiculi of 2 μm. Notes with the type material described spores up to 40 μm, but I have not seen spores longer than 33 μm. Mexican material has spores of (23-) 25-33 x (8-)10-12 μm, with longer apiculi (up to 2.5 μm) than as noted in the type specimen. The species was described in detail by Abbott and Currah (1997) and Harmaja (1969b) from the United States and Europe. These collections represent the first record of *G. ambigua* from Mexico. This species grows in soil or wood in *Abies religiosa* and *Pinus hartwegii* forests, at altitudes of around 2900 m.

Studied material: MEXICO. Michoacan. Villa Madero Municipality, Cerro Cruz Gorda, highway Pátzcuaro-Tacámbaro, 23/X/1979, *Sánchez 8* (XAL). MORELOS. Lagunas de Zempoala, road to Chalma, 12/X/1965, *Díaz y Carmona* s.n., (XAL, ENCB). CANADA. Prince Edward Island, Herb Ellis (FH). FINLAND. Tammela, 30/VII/1866, *Karsten 3289* (holotype of *H. infula* var. *similis*, H). UNITED STATES. Michigan, /IX/1979, *Beardslee* s.n. (FH).

Gyromitra esculenta (Pers.: Fr.) Fr., Summa VegetabiliumScandinaviae 2: 346. 1849. **Fig. 3-4**

≡ *Helvella esculenta* Pers.:Fr., Commentarius Schaefferi icones pictas p. 64. 1800.
= *Gyromitra bubakii* Velen., Ĉeske houby p. 893. 1922 (fide Abbott and Currah, 1997).

The brain-like apothecia with vinaceous brown, reddish or violaceous tones are typical of this species. There is a great deal of variability, as reported by Harmaja (1979a), in *G. esculenta*, including hymenium folding, apothecial margins, color of the hymenium and stipe. Harmaja attributes this variation primarily to environmental factors and maturity of the material. He considered there to be three spores types within this group: type I (*G. esculenta* s. str.), spores 18-23 (-25) x 10-12.5 μm, elliptical to fusiform with apiculi up to 0.5 μm; type II (without any taxon assigned), spores 20-25 x 10-12.5 μm, subfusiform, apiculi up to 1 μm, and type III (*G. splendida* and *G. bubakii*?), spores 22-30 x 10-12.5 μm, fusiform, apiculi 0.6-1.2 μm.

I regard *G. esculenta* to be a species complex that includes *G. bubacii, G. esculenta* s. str., *G. longipes* Harmaja and *G. splendida* Raitv. All of these have very similar morphology and spore size. Raitviir (1974) described *G. splendida* with deep violaceous tones in the hymeniun and stipe, with a stipe longer than in *G. esculenta*, and subfusiform spores 23-28 x 11.5-13.5 μm, with a perisporium thickened up to 2 μm. This author pointed out that the long stipe and not so broad cap were different than *G. esculenta*, but in the field these species were indistinguishable. Harmaja (1979b) considered *G. longipes* to differ from *G. esculenta* s. str., in its darker pileus and long stipe with violaceous tones and in its fusiform spores 20-25 x 9-10 μm, with apiculi up to 2 μm. Huhtinen and Ruotsalainen (2004), studying specimens from Finland, suggested the conspecificity of *G. splendida*

Table 1. Characters of the species *G. esculenta* complex

	Apothecium	Spore shape	Size (μm)	Apiculum
G. esculenta (1)	gyrose, vinaceous brown, reddish to violaceous tones stipe more or less long*	ellipsoid-	18-23 (-25) x 10-12.5	0.5μm
G. esculenta (II)		subfusiform	20-25 x 10-12.5	0.5-1μm
G. esculenta (III) = *G. splendida*, *G. bubakii*?		fusiform	22-30 x 10-12.5	0.5-1.5μm
G. longipes	lightly gyrose, dark color with violet tones, long stipe	subfusiform	20-25 x 9-10	1-2μm
G. splendida	gyrose, dark- brown to almost black with violet tones, long stipe	subfusiform	23-28 x 11.5-13.5	1-2 μm

*'long' = 2/3 of the total length of the ascocarp

and *G. longipes*. In fact, regarding morphological characters and measurements of spores (type III of Harmaja), *G. longipes* and *G. splendida* seem to be the same (see Table 1). *G. bubakii* (Velenovsky, 1922) has smaller fruitbodies, longer stipes (2/3 of the total length) and bigger spores 30-34 μm than *G. esculenta*. The type of *G. bubakii* was study by Moravec (1986), Abbott and Currah (1997) and Huhtinen and Routsalainen (2004). For the former *G. bubakii* is a variety of *G. esculenta*, while for Abbott and Currah it is conspecific with *G. esculenta*, and Huhtinen and Routsalainen considered it different from *G. esculenta*. Moravec (1986) showed a continuous wall on the spore, but not in the apiculi. The loss of apiculi is attributed to preservation in formaldehyde (Huhtinen and Rouatsalainen 2004), but Kempton and Wells (1973) have suggested that spores of *G. esculenta* four weeks old, lose their apiculi. Spores of *G. esculenta* seems to vary widely in size. Most of the material studied from Mexico has elliptical to subfusiform spores 18-20 x (8-) 10-12.5 μm, which correspond to type I. Nine of the studied specimens presented subfusoid spores of 20-25 (-27) x 10-12.5 μm with apiculi up to 1 μm (type II) and only one specimen (*Medel 11*, XAL) had very large fusoid spores, 27-33 x 11.8-13.5 μm, with apiculi of more than 1 μm. Mexican material shows great variability in ascoma shape, length and width of the stipe, but not in ascoma color. This variability was similar to that found in the materials from Canada, England, Finland and the United States. To understand this variability it will be necessary to study more material of *G. esculenta* from the whole geographical range, particularly in order to judge the importance of spore size in the taxonomy of the group. Regarding Mexican materials *G. esculenta* s. str. is considered to have the following characters: spores of 18-20 (-27) x (8-)10-12.5 μm, folded brain-like hymenia that are vinaeous brown, reddish or violaceous tones, a stipe, elliptical spores, and apiculi up to 1 µm. This measurement falls in both spore type I and II of Harmaja and coincides with the measurements cited by diverse authors (Boudier 1905, Dennis 1978, Kempton and Wells 1973, De la Torre 1977, Weber 1988, Abbott and Currah 1997). Moravec (1986) found that the spores in *G. esculenta* have a wide continuous range of variation up to 29.5 µm. I have not seen Mexican material with spores longer than 27 µm. Among the studied specimens, one of the collections (*Medel 11*, XAL) could represent another taxon, because of the subfusiform spores 27-33 x 11.8-13.5 µm with apiculi of 1-1.5µm.

At present there is no consensus about *G. esculenta* spore size. Moravec (1986) suggested that the original description of *G. esculenta* by Persoon was based on immature specimens. Harmaja (1979a) noted that the original concept of *Helvella esculenta* needed clarification and he mentioned several names that he thought were synonyms of *G. esculenta.* Abboth and Currah (1997) mentioned the type specimen is unknown, and so a neotype should be selected. Full descriptions of *G. esculenta* can be found in De la Torre (1977), Abbott and Currah (1997), Kempton and Wells (1973) and Weber (1988). The species is humicolous and lignícolous, and grows in *Abies religiosa, Pinus, Pinus-Quercus* and rarely in cloud forest at 2500-3600 m.

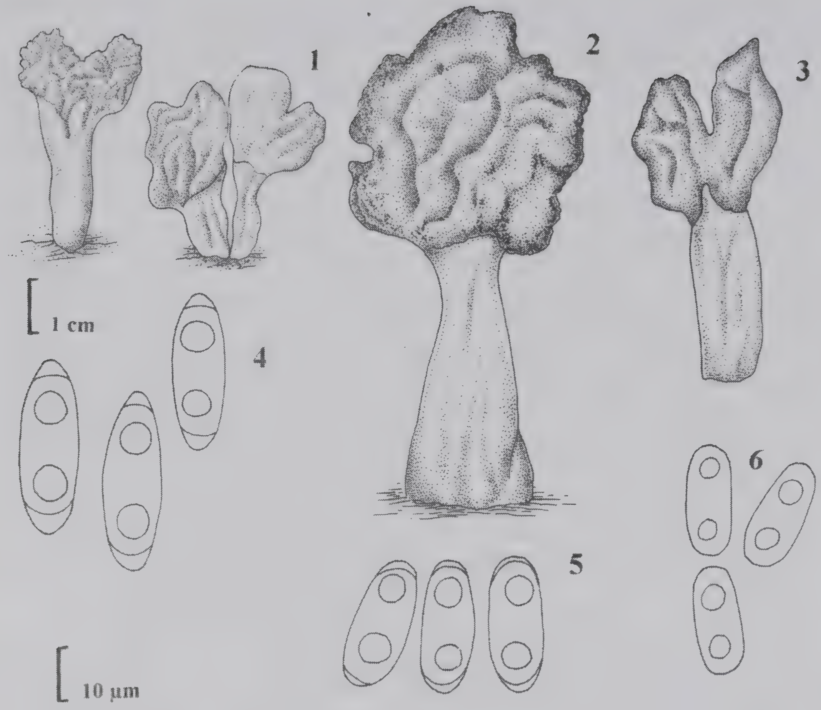
Studied material: MEXICO. COAHUILA. Arteaga Municipality, Las Carolinas, 27/VII/1986, *García* s.n. (ITCV); Arteaga Municipality, La Siberia, 19/X/2001, *Medel* s.n. (FCF). DURANGO. Highway Durango-Mazatlán, 28/VII/1984, *Garcia 434* (ITCV). ESTADO DE MÉXICO. Los Saucos, deviation to Valle de Bravo, highway Toluca-Temascaltepec, 15/VIII/1982, *Chacón 345* (ENCB); National Park Lagunas de Zempoala, highway to Chalma, 1/VIII/1982, *Chacón 287* (ENCB); Villa de Allende Municipality, San Cayetano N of Agua Escondida, 5/IX/1982, *González 42 (*ENCB); West side Paso de Cortés, Popocatepetl Volcano, 26/IX/1986, *Chacón 570* (ENCB); Escualango, East side of San Rafael Atlixco, 7/X/1983, *Hernández 136* (ENCB). GUERRERO. Chilpancingo de los Bravo Municipality, Cerro Palo Hueco, Omiltelmi, *14/8/1984, Gutierrez* s.n., (FCME); Km 2.5 desviation to Puerto del Gallo, 27/VIII/1983, *Tapia* s.n., (FCME). HIDALGO. National Park El Chico Sierra de Pachuca, 31/10/1981, *Ramos* s.n. (ENCB); El Chico 13/8/1965, *Salas* s.n., (ENCB). MICHOACÁN. Zinapécuaro Municipality desviation to Eréndira, San Pedro Jacuaro, *Hernández y Villegas 924* (FCME).TLAXCALA. National Park La Malinche, 15/IX/1970, *Rodríguez-Martínez 43* (ENCB). VERACRUZ. Xico Municipality, Los Gallos, East side Cofre de Perote, *Rico 951* (XAL); Xico Municipality, near El Llanillo road Las Vigas-Tembladeras North side Cofre de Perote, *Bandala 2654* (XAL*);* Calcahualco Municipality, near Tlacoteopa, road La Jicara *Medel 891, 892, 893, 894, 895* (XAL); Cofre de Perote region, road Valle Alegre Park to Tembladeras, *Ramírez-Guillén 222* (XAL); IV Exposición de Hongos del INIREB, 31/VIII/1986, *Medel 11* (XAL); Cofre de Perote region, Valle Alegre Park, 13/VIII/2003, *Guzmán 35515* (XAL).

Gyromitra infula (Schaeff.: Fr.) Quél, Enchiridion Fungorum 272, 1886. Fig. 5-6

≡ *Helvella infula* Schaeff.: Fr., Fungorum qui in Bavaria et Palatinatu nascuntur icones 4:105. 1774.

= *Helvella friesiana* Cooke, Mycographia seu icones Fungorum 1: 195, 1878.

The bilobate or saddle-shape, brown-orange apothecia and elliptic non-apiculate spores, 17.6-25 x 7.2-10 µm, are characteristic of *G. infula*. This species has the widest distribution of all the species reported in Mexico and is morphologically close to *G. ambigua.* Spore size and the lack of apiculi are diagnostic features. Our material has spores of 17.6-20



Figs. 1-6. Figs. 1-2. *Gyromitra ambigua.* 1: ascoma; 2: spores (showing apiculum). Figs. 3-4. *G. esculenta.* 3: ascoma; 4: spores (with a short apiculum). Figs. 5-6. *G. infula* 5: ascoma; 6: spores

(-25) x 7.2-10 µm. This is smaller than reported in the neotype specimen of *G. infula* (designated by Harmaja, 1969b) that is also the lectotype of *H. friesiana* at K, in which the spores reach a size of 20-25 x 8-10 µm, according to my observations. Additional descriptions of this species can be found in Abbott and Currah (1997), Kempton and Wells (1973), De la Torre (1979) and Weber (1988). The species grows on wood, humus or on burned soil, in forests of *Abies religiosa*, *Pinus*, and *Pinus-Quercus* and rarely in cloud forests, at 2700-3500 m.

Studied material. MEXICO. COAHUILA. Arteaga Municipality, La Siberia, 15/8/1973, *Guzmán 11248, 11251* (ENCB), 21/IX/1980, *Chacón 98* (ENCB). DISTRITO FEDERAL. El Ajusco, near Pico del Aguila, 25/IX/1966, Fagoaga 15c (ENCB). DURANGO. Pueblo Nuevo munipality, El Mil Diez, *Garcia 3470* (ITCV). ESTADO DE MEXICO, highway Toluca-Temascaltepec, deviation to Valle de Bravo, 23/X/1986, *Santillán 540* (ENCB); Texcoco Market, 8/IX/1973, *Velásquez 832* (ENCB); El Capulín, highway to Sultepec, Nevado de Toluca, Nacional Park, 25/9/1983, *González 458* (ENCB); Nevado de Toluca, highway to Sultepec, 2/IX/1983, *Colón 358* (ENCB); Nevado de Toluca, cerro El Calvario, 24/IX/1983, *Colón 378-A,* 382 (ENCB); Villa Nicolás Romero Market, 17/10/1976, *Baca s.n.* (ENCB); Laguna de Ojotongo, highway Zempoala-

Chalma, 15/VII/1963, *Guzmán 5086*, (ENCB); Río Frío near Llano Grande, highway México-Puebla, 13/VII/1968, Díaz 9, Guzmán 1538 (ENCB); Temoaya Municipality, Las Navajas, Kong-Luz 54 (ENCB) GUANAJUATO. El Zamorano, near Tierra Blanca, 27/VIII/2000, *Landeros 7-10B* (XAL). MORELOS. Lagunas de Zempoala, *Rocha 10* (ENCB); Huitzilac Municipality, Lagunas de Zempoala National Park, 26/7/1982, *Aguilar s.n.* (FCME). GUERRERO. Chichihualco Municipality, between Carrizal and Puerto del Gallo, 12/7/1980, *Lugo s.n.*, 12/VII/1980, Villarías 52, 20/VIII/1983, *Perez-Ramírez 467*, *Herrera s.n.* (FCME); Chilchihualco Municipality, between El Carrizal and Atoyac, *Lugo 18* (FCME), Chilpancingo de los Bravo Municipality, Palo Hueco, Omiltelmi, 14/8/1984, *Gutierrez s.n.* (FCME). MICHOACÁN. Ciudad Hidalgo Municipality, National Park Cerro Garnica, 18/8/1983, *Arrieta s.n.* (FCME). MORELOS. Cuernavaca, region of the Lagunas of Zempoala, 19/VIII/1982, *Mora 429*, *Chacón 3613* (XAL). NUEVO LEON. Zaragoza Municipality, La Encantada, *García 2414, 8195* (ITCV); Zaragoza Municipality, El Viejo Hill, García 2583 (ITCV): OAXACA. Highway Tuxtepec-Oaxaca, between La Esperanza and Oaxaca, 25/VII/1977, *Pérez-Ortíz 695,* (ENCB). PUEBLA. Chiautzingo Municipality, San Juan Tetla, Cañada Chamier, 27/10/1982, *Gauzín s.n.* (ENCB); South of Hidalgo, Medel 680 (XAL). VERACRUZ. Municipality de Xico, West side Cofre de Perote, Los Gallos near El Rosario, *Rico 934, 949, Montoya 240, Bandala 465, Pérez-Moreno 397, 477, Villarreal 1334, 2191, 2618, 2580, 2562, 2618* (XAL); Xico Municipality, El Rosario near El Revolcadero, *Montoya 330-A, 381, Bandala 357, 406,464, Villarreal 631-A, 646,664,1465, 1546, 2251* (XAL), Calcahualco Municipality near Tlacoteopa, La Jícara road, 10/X/1998, *Medel 890* (XAL). SWEDEN. Uppsala (ex Herb. Berkeley lectotype of H. friesiana, K).

Acknowledgements

The author expresses her thanks to the following: Dr. Gastón Guzmán (Instituto de Ecologia at Xalapa), Dr. Donald Pfister and Dr. Karen Hansen (Farlow Herbarium, Harvard University) for critical reading the manuscript. Dr. Francisco Lorea and Donald Pfister improved the English, Juan Lara Carmona helped in work in the Herbarium, Manuel Escamilla made the drawings and Maricruz Peredo for the edition of plates. CONACYT provided financial support through a grant. I also benefited from a stay at the Farlow Herbarium Harvard University where I was supported by a fellowship provided by the Friends of the Farlow. Also I express my thanks to the Curators of the following Herbaria: ENCB, FCF, FCME, FH, H, ITCV and K for the loan of specimens for study.

Literature cited

Abbott SP, Currah S. 1997. The *Helvellaceae:* Systematic revision and ocurrence in Northern and Northwestern North America. Mycotaxon 62: 1-125.

Boudier E. 1905. Icones mycologicae ou iconographies des champignons de France, Paris.

Castillo J, García J, San Martín FE. 1979. Algunos datos de la distribución ecológica de los hongos principalmente micorricicos, en el centro del Estado de Nuevo León. Bol. Soc. Mex. Mic. **13**: 229-237.

De la Torre M. 1977. Aportación al estudio del genero *Gyromitra* Fr. em. Harmaja en España. Bol. Estación Central de Ecología 19: 33-38

Dennis RWG. 1978. British Ascomycetes. Cramer, Vaduz. 585 pp.

Eckblad FE. 1968. The genera of the operculate Discomycetes: A re-evaluation of their taxonomy, phylogeny and nomenclature. Nyt. Mag. Bot. 15: 1-191.

Guzmán-Dávalos L, Ramírez O, Sánchez-Jácome MR, Chacón S. 2001. Ascomycotina conocidos de Jalisco. Boletín IBUG 9: 11-23.

Harmaja H. 1969a. A wider and more natural concept of the genus *Gyromitra* Fr. Karstenia 9: 9-12.

Harmaja H. 1969b. A neglected species *Gyromitra ambigua* (Karst.) Harmaja n. comb. and *G. infula* s. str. in Fennoscandia. Karstenia 9: 13-19.

Harmaja H. 1973. Amendments to the limits of the genus *Gyromitra* and *Pseudorhizina,* with the descriptions of a new species *Gyromitra montana.* Karstenia 13: 48-58.

Harmaja H. 1979a. Notes on *Gyromitra esculenta* and *G. recurva*, a noteworthy species on western North America. Karstenia 19: 46-49.

Harmaja H. 1979b. Studies on vernal species of *Gyromitra* and *Pseudombrophila* syn. *Nannfeldtiella.* Ann. Bot. Fennici 16: 159-162.

Herrera T, Guzmán G. 1961.Taxonomía y ecología de los principales hongos comestibles de diversos lugares de México. Ans. Inst. Biol. UNAM. 32: 33-135.

Huhtinen S, Ruotsalainen J. 2004. Notes on the taxonomy and ocurrence of some species of *Gyromitra* in Finland. Karstenia 44: 25-34

Kempton PE, Wells VL. 1973. Studies in the fleshy fungi of Alaska VI. Notes on *Gyromitra.* Mycologia 65: 396-400.

Kimbrough J, Chi-Guang W, Gibson JL. 1990. Ultrastructural observation on *Helvellaceae* (*Pezizales,* Ascomycetes) IV. Ascospore ontogeny in selected species of *Gyromitra* subgenus *Discina.* Can. J. Bot. 68: 317-328.

Kimbrough J. 1991. Ultrastructural observations on *Helvellaceae* (*Pezizales,* Ascomycetes). V. Septal structures in *Gyromitra.* Mycol. Res. 95: 421-426.

Kirk PM, Cannon PF, David JC, Stalpers JA. 2001. Ainsworth & Bisby´s Dictionary of the Fungi. 9a. Ed., CABI Biocience, Surrey, 655 pp.

Korf RP. 1972. Synoptic key to the genera of *Pezizales.* Mycologia 64: 937-994.

Mcknight KH, Batra L. 1974. Scanning electron microscopy in taxonomy of gyromitroid fungi. The Michigan Botanist 13: 51-64

Moravec J. 1986. A revision of the type of *Gyromitra bubaci* and the problem of ascospore size of *Gyromitra esculenta* (Discomycetes). Ceska Mykologie 40: : 11-18

O'Donnell K, Cigelnik E, Weber N, Trappe J. 1997. Phylogenetic relationship among ascomycetous truffles and the true and false morels inferred from 18s and 28s ribosomal DNA sequence analysis. Mycologia 89: 48-65.

Pfister DH. 1980. *On Peziza melaleucoides*, a species of *Gyromitra* from the western United States. Mycologia 72: 614-619.

Raitviir A. 1965. Taxonomical notes on the genus *Gyromitra.* Eesti NSV Teaduste Akadeemia Toimetised (Bioloogiline Seeria 14(3): 320-324.

Raitviir A. 1974. A new species of *Gyromitra* from Estonia. Folia Cryptogamica Estonica 4: 25-32.

Saccardo PAM. 1889. Sylloge Fungorum VIII. Discomycetae. J. Edwards, Ann Arbor. Michigan, 1143 pp.

Seaver FJ. 1928. The North American Cup-Fungi (Operculates). Reiprint, 1978. Lubrecht & Cramer, Monticello, 377 pp.

Velenovsky J. 1922. Ĉeske houby 4-5: 633-650.

Weber NS. 1988. A morel hunter´s companion. A guide to the true and false morels of Michigan. Michigan Natural Resources Magazine. Two Peninsula Press, Lansing 208 pp.

MYCOTAXON

Volume 94, pp. 111–125 October–December 2005

A new species and a new record of *Lepiota* occurring in the Gulf of Mexico area

Leticia Montoya & Victor M. Bandala

montoya@ecologia.edu.mx bandala@ecologia.edu.mx
Biodiversidad y Sistemática, Instituto de Ecología
P.O. Box 63, Xalapa, Veracruz 91000, Mexico

Abstract — Two species of *Lepiota* are described from material collected in the central area of Veracruz State, viz. *L. trichroma,* a new species proposed here, and *L. hemisclera,* an unreported species from Mexico. Samples of both taxa were found in the cloud forest (mesophytic or subtropical forest) surrounding Xalapa. Descriptions and discussions, including illustrations of macro- and microscopic morphology of both taxa based on the Mexican collections are provided. Microscopical observations after studying the lectotype of *Agaricus hemisclerus* from Cuba, as well as material of *Lepiota subcitrophylla* from Japan are included.

Key words — *Agaricaceae, Stenosporae*

Introduction

During recent fieldwork to monitor the macromycetes in the central region of Veracruz state, several collections of two species of *Lepiota,* previously unrecorded in the region, were found. One of these species, *L. trichroma,* presents a combination of macro- and micromorphological characters that places it in an undescribed taxon belonging to the group of spurred-spored species close to *L. bichroma* E. Horak, *L. subcitrophylla* Hongo, and *L. citrophylloides* Sathe & S. M. Kulk. The other species corresponds to *L. hemisclera* of which the present collections document the information of its morphological variation and distribution range. The two *Lepiota* species studied were found at Santuario del Bosque de Niebla (cf. Parque Ecológico Fco. J. Clavijero in previous publications) and the native wooded area of the Botanical Garden Francisco Javier Clavijero, both corresponding to a cloud forest (subtropical or mesophytic forest according to Rzedowsky 1978) protected by the Instituto de Ecología (Xalapa), consisting of a mixed association dominated by *Quercus* spp., *Carpinus caroliniana* Walter, *Clethra mexicana* DC., *Ostrya virginiana* (Mill.) K. Koch and *Liquidambar styraciflua* L. var. *mexicana* Oersted, which has been visited weekly throughout the year since 2002.

Materials and Methods

Macroscopic descriptions are based on the study of fresh material. Alphanumeric color codes indicated in the descriptions refer to Kornerup and Wanscher (1967) and Munsell

(1994) (bold codes). Microscopic structures are described from hand sections of revived tissues mounted in 3% KOH and Congo Red 1% aqueous solution. Measurements and colors of the structures were observed in 3% KOH. The basidiospores were measured in side view, 30 spores randomly selected from hymenophoral tissues were evaluated in a single element per collection. When estimating spore dimensions part of the recommendations by Heinemann and Rameloo (1985) were used. The first range reported in the descriptions describes the variation within the collections and extreme values are indicated in brackets (highest values of mean ±2 SD in each sample). The means of spore length, width and quotient Q (ratio of basidiospore length/basidiospore width) were calculated in each collection, then $\overline{x}$ corresponds to the range of means of length and width, and **Q** to the range of the mean values of Q of **n** collections. Line drawings were made with the aid of a drawing tube. Herbarium acronyms are according to Holmgren et al. (1990).

Taxonomy

Lepiota trichroma Montoya & Bandala **sp. nov.**

FIGURES 1-3

> ***Pileus*** *8–35 mm, convexus vel plano-convexus, interdum tenui umbonatus, pallide flavovirens, caerulescens, saepe rubigineomaculatus, subglaber vel minute subsquamulosus (sub lente), squamulis obscurioribus.* ***Lamellae*** *liberae, confertae vel subdistantes, flavidae, saepe rufomaculatae, caerulescens.* ***Stipite*** *22–70 × 1–4 mm, cylindricus, pileo concolor, caerulescens et rufomaculatus, fibrillosus vel minute subsquamulosus, squamulis obscurioribus, ad basim interdum rhizomorphis rubescens observatis.* ***Cortina*** *fibrillosa, mox destituta, interdum fibrillis apud annulus zonis instructo, raris ad pileus margin.* ***Caro*** *flavidae, caerulescens, rufo maculatae (stipite).* ***Basidiosporae*** *6–8.5 (–9) × 3–4 (–4.3) µm, elongatae, leves, calcaratae.* ***Pleurocystidia*** *nulla.* ***Cheilocystidia*** *13.5–30 × 6–12 µm, clavata, ventricosa, vel utriforme.* ***Pileipellis*** *ex hyphis cilindraceis cutem formantibus, elementa terminalis clavata, decumbentes vel ascendentibus, dispersus, in squamules saepe inflata pileocystidia similis, 7–40 × 8–25 µm, cumulus formantibus.* ***Fibulae*** *presentes. MEXICO, Veracruz, Xalapa Co., km 2.5 via Xalapa-Coatepec, Instituto de Ecología, Santuario del Bosque de Niebla, 3 July 2003, Bandala 3776 (XAL Holotypus).*
>
> ***Etymology:*** Because of the yellow basidiomes which stain reddish and blue.

Pileus 8–35 mm diam., convex to plane-convex, sometimes weakly umbonate, occasionally depressed at center, at first faintly tomentose, becoming variably minutely squamulose (under lens) especially in the center, smooth or slightly rugulose, dry, pale yellow (2A4, 4B5), lemon-yellow (1-2A4, 30A4-5), or sulphur-yellow (2B5, 30A3-4) (**5Y 8/6-8**), caerulescent (especially on handling), with pale pinkish tinges, finally with red or orange-oxide scattered stains, squamules brownish on yellow ground, these in groups giving a dark (brown) coloration to the disc center; margin straight, entire or somewhat eroded in age, obscurely striate, occasionally in parts bearing minute, fine fibrillose veil remnants. ***Lamellae*** close to subdistant, rounded-free or faintly adnate, at times rather seceding, subventricose, 3–4 (–5) mm broad, yellow (2A2, 3A4-B7) to sulphur-yellow (30A3-A4), caerulescent, frequently with red or orange-oxide stains, finally stained dark brown, edge entire, somewhat paler than the surfaces; lamellulae up to 4 different lengths.

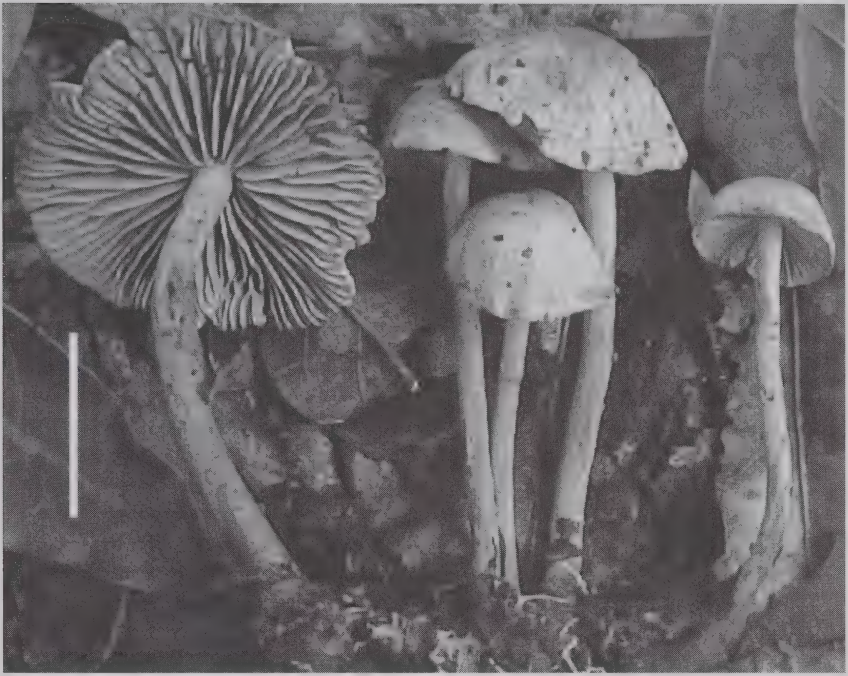


Fig. 1. ***Lepiota trichroma.*** Basidiomes (*Bandala 3776*). Bar = 17 mm.

Stipe 22–70 × 1–4 mm, cylindric, straight, often slightly curved downwards, yellow (2A2-2B5, 3A4, 4B4), concolorous with pileus, often with a brighter sulphur-yellow color near lamellae attachment, commonly stained blue (or when handling), whilst other areas irregularly stained red or reddish-brown to orange-oxide, finally becoming brownish or dark brownish spotted; apex pruinose, fibrillose to minute squamulose downwards (under lens), squamules scattered, darker; partially fistulose, filled with whitish fibers; base concolorous, at times stained orange-oxide, more or less strigose by the presence of white to pale yellowish mycelium, this often forms moderately coarse rhizomorphs. ***Veil*** fibrillose, soon disappearing with age, occasionally as scant fibrils remaining at annular zone or rarely at pileus margin. ***Context*** yellow, hygrophanous, caerulescent, stained reddish-brown or reddish mainly in the inferior two thirds of stipe including the rhizomorphs. ***Odor*** to rotten potatoes or herbs. ***Taste*** mild.

Basidiospores 6–8.5 (–9) × 3–4 (–4.3) µm; $\overline{x}$ = 7.1–7.6 × 3.3–3.5 µm, ***Q*** = 2.1–2.2, elongate, spurred on lateral view and with a dorsal depression, pale yellowish-green to hyaline, wall slightly thick, dextrinoid. ***Basidia*** 15–25 × 5–6.5 µm, clavate, bi- or tetrasporic, thin walled, hyaline. ***Pleurocystidia*** absent. ***Cheilocystidia*** 13.5–30 × 6–12 µm, clavate, broadly clavate or more or less narrowly utriform, often somewhat ampulliform, hyaline, thin walled, numerous. ***Pileipellis*** a cutis composed of interwoven, cylindric hyphae 4–7 µm broad, often ramified, clamped, at times ending in a projected or periclinally oriented claviform element, often the scales with mounds of compactly arranged clavate,

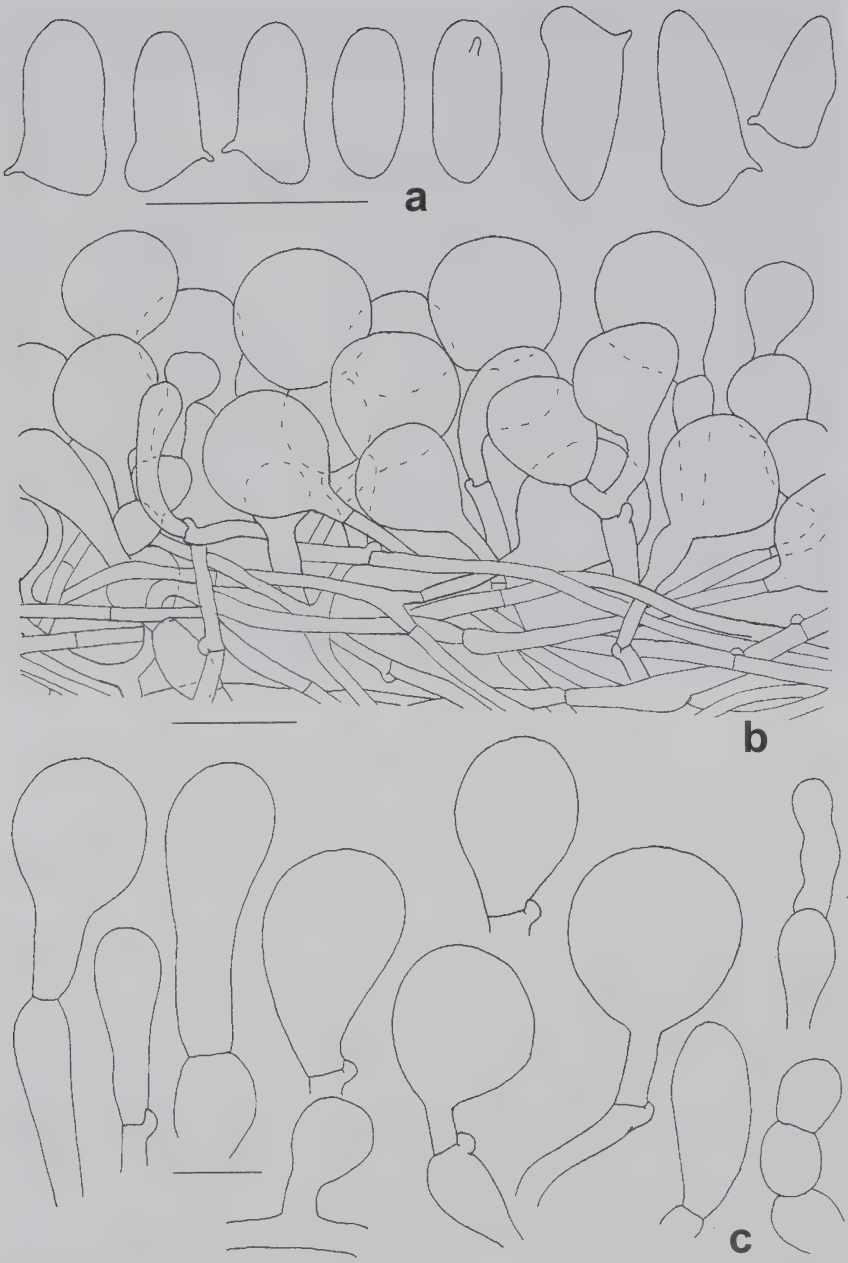


Fig. 2. ***Lepiota trichroma.*** a: basidiospores; b: elements of the pileipellis and scales; c: scale elements (*Bandala 3776*). Bar a, c = 10 µm, b = 20 µm.

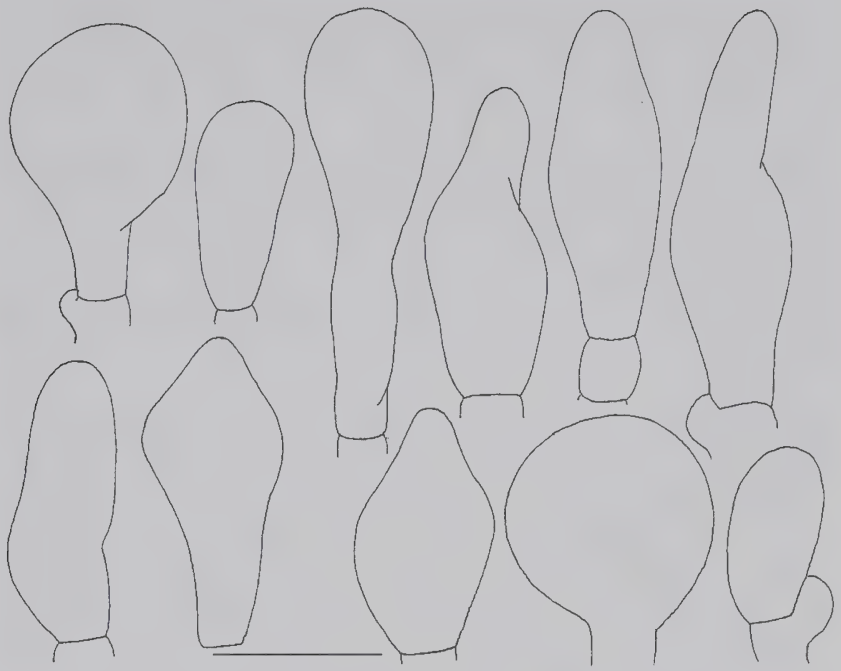


Fig. 3. ***Lepiota trichroma.*** Cheilocystidia (*Bandala 3776*). Bar = 10 µm.

broadly clavate or sphaeropedunculate pileocystidia-like elements, 7–40 × 8–25 µm, 4–7 µm diam. at base, some more or less disposed in chains of 2–3 subisodiametric cells, all terminal elements with yellowish wall and with yellowish-brown contents, slightly thick walled (up to 0.5 µm thick), at times with incrustations. ***Context*** hyphae 4–13 µm diam., yellowish to yellowish-brown, thin walled, clamped. ***Hymenophoral trama*** subregular, hyphae 2.5–18 µm diam., cylindric to inflate, broad or sausage shape. ***Stipe trama*** with somewhat parallel hyphae 5–8 µm diam., thin or somewhat thick walled, yellowish in KOH, clamped. ***Stipitipellis*** a cutis of hyphae 4–8 µm diam., at times ending in a sphaeropedunculate element 19–40 × 8–12 µm or a subisodiametric cell up to 30 µm diam., other hyphae with short lateral outgrowths, clamped. ***Clamp connections*** present. A yellow sap is dissolved in KOH slides.

Habitat. Solitary to gregarious, at times caespitose, in small troops, on soil or among fallen leaves or grass, near *Carpinus caroliniana,* at 1300 m.

SPECIMENS EXAMINED — MEXICO. VERACRUZ: Xalapa Co., km 2.5 old road Xalapa-Coatepec, Instituto de Ecología, Santuario del Bosque de Niebla, 26.VI.2002, *Corona 68*; 21.X.2002, *Bandala 3714;* 25.X.2002, *Montoya 3958;* 14.XI.2002, *Corona 125;* 26.VI.2003, *Corona 263, 264;* 3.VII.2003, *Bandala 3776* (*Holotype,* XAL). Instituto de Ecología, Botanical Garden Francisco J. Clavijero, native wooded area, 4.VII.2002 *Montoya 3837, 3838;* 27.IX.2002, *Montoya 3922;* 13.VII.2005 *Bandala 3981* (all at XAL).

Other material studied — *Lepiota subcitrophylla.* JAPAN. Ooshimizu, Ibuki-sho, Shiga Pref., on soil in *Pinus densiflora-Quercus serrata* forest, 1.IX.1973, leg. *Z. Sugiyama* (*TMI 1458*); Kokoge, Tottori City, Tottori Pref., on soil in mixed Wood of *Camellia, Cinnamomum, Aphananthe, Phyllostachys,* etc., 12.XI.1974, leg. *E. Nagasawa* (*TMI 1847*).

Remarks. *Lepiota trichroma* is readily recognized by its yellow, caerulescent basidiomes developing pale pinkish, red or orange-oxide stains, the spurred spores, broadly clavate or ventricose cheilocystidia and pileipellis irregularly covered with subisodiametric or sphaeropedunculate terminal elements forming the scales of the pileus. The microscopic characteristics (pileipellis structure, basidiospores, cheilocystidia) in combination with color variation of the basidiomes, taxonomically separate *L. trichroma* from phenotypically similar taxa in Section *Stenosporae* (J. Lange) Kühner (Bon 1981; Candusso & Lanzoni 1990; Vellinga & Huijser 1993).

The blueing of the basidiomes is a characteristic rarely found among the species of *Lepiota* with spurred spores (Akers et al. 2000; Horak 1980). *Lepiota subcitrophylla* described from Japan (Hongo 1956) and depicted in Imazeki and Hongo (1983) iconography, superficially recalls *L. trichroma.* The distinctive reddish colors of the basidiomes as observed in the Mexican taxon, however were not described for *L. subcitrophylla* (Hongo 1956; Imazeki and Hongo 1983) and there are also taxonomically important morphomicroscopical differences. A re-examination of two Japanese collections of this latter species (one of them, *TMI 1458,* determined by Hongo), revealed a pileipellis of a trichodermis kind, composed of a disrupted layer of elongated (narrowly claviform, subcylindric or narrowly lageniform) terminal elements 35–145 (–160) × 6–14 (–16) µm, slightly bigger basidiospores [7–10 (–11) × 3–4 (–4.5) µm, $\bar{x} = 8.7 \times 3.3$ µm, $Q = 2.67$] and lamellae edges lacking cheilocystidia (fig. 4). Hongo (1956) reported, however, cheilocystidia crowded, clavate to subcylindric or somewhat fusoid, 19–31 × 6–14 µm and dermatocystidia cylindric to clavate, 35–70 × 9.5–16 µm.

When describing the Australian *L. bichroma,* Horak (1980) observed a resemblance between it and *L. subcitrophylla.* The basidiomes of *L. bichroma,* as emphasized by Horak, are not caerulescent, instead they are distinguishable by the deep lilac colors produced by minute squamules or wart-like squamules of the pileus and stipe, respectively, on a pale yellow-orange background, therefore differing macroscopically from the Japanese taxon and the Mexican *L. trichroma.* Furthermore, microscopically *L. bichroma* differs from *L. trichroma* in its longer basidiospores (9–12 × 3–4 µm) and the pileipellis characteristics (a palisade of clavate to subfusoid elements 30–80 × 5–16 µm, having a pale brown encrusting and plasmatic pigment) (Horak 1980). Another species close to *L. trichroma* is *L. citrophylloides* described from India (Sathe and Kulkarni 1980). Regrettably, it has not been possible to study the holotype housed at AMH. Based upon descriptive data provided by Sathe and Kulkarni (1980) *L. citrophylloides* can be separated from the Mexican taxon by its apparently not caerulescent basidiomes lacking reddish tinges and the umbonate pileus with olivaceous brown scales, white veil, crowded lamellae and cylindric to narrowly clavate cheilocystidia 21–27 × 6–7.5 µm.

The monitoring developed in a square plot of 640 m^2 in the cloud forest of Santuario de Niebla showed that during 2002 to 2003 the basidiomes of this taxon occurred in 6

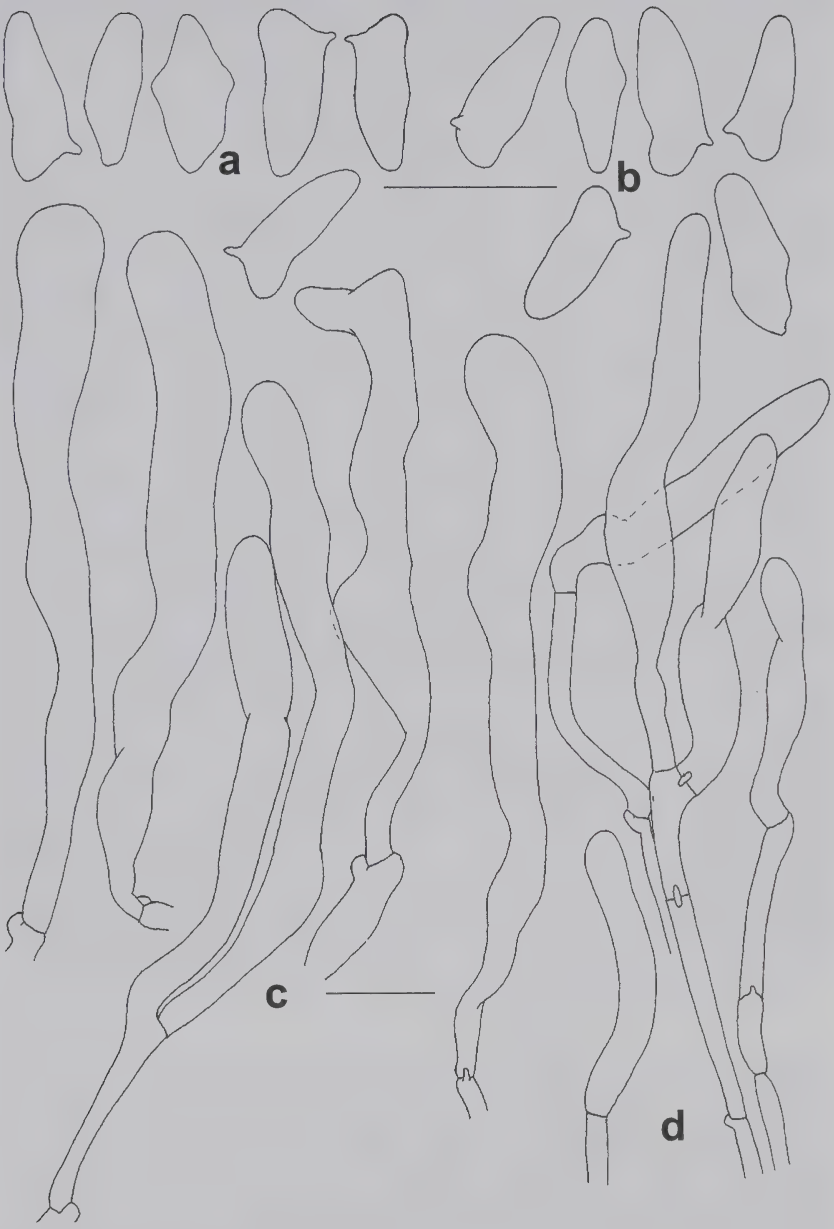


Fig. 4. ***Lepiota subcytrophylla.*** a-b: basidiospores; c-d: pileipellis elements (a, c: *TMI 1847;* b, d: *TMI 1458*). Bar a-b = 10 µm; c-d = 20 µm.

subsquares (each of 1 m^2), growing from solitary to moderately gregarious. It presented a summer fruiting pattern, at times reaching the fall. Although the monitoring continued throughout 2004-2005, it was recorded again until September 2005, only in the Botanical Garden area.

Lepiota hemisclera (Berk. & M.A. Curtis) Sacc., Syll. Fung. 5: 66, 1887.
= *Agaricus hemisclerus* Berk. & M.A. Curtis, Jour. Linn. Soc. Bot. 10: 283, 1868

FIGURES 5-8

Pileus 40–75 mm diam., subglobose to subhemispheric when young, gradually convex, becoming plane-convex or plane, moderately umbonate, at times finally almost plane-concave, dry, somewhat hygrophanous, surface weakly wavy, with darker lines arranged in a striking reticulate pattern on all the surface, the paler ground areas yellow-brown to straw or wood yellow color (**10YR8/6; 7/6**), with the reticulum brownish, brownish-orange or grayish-orange (5C5-6, 5B5); surface covered with abundant pyramidal to echinate, blackish-brown, detersile scales, located often inside the mesh and distributed in a more or less concentric pattern throughout the pileus, with the pileus expansion they are more persistent towards the disc center where the mesh is denser, then this area appears more uniformly brownish-orange or at least darker; the darker lines glabrous in appearance but under lens faintly minutely fibrillose; margin at first more or less inflexed, becoming straight, often undulate (especially in very expanded pilei), not striate, at times minutely appendiculate in parts by veil remnants. ***Lamellae*** free, crowded, initially arcuate in unexpanded pileus to subventricose, at times rather linear, moderately narrow, 4–5 mm broad, some forked, with lamellulae of different size, whitish or yellowish-white, edge concolorous, somewhat irregular. ***Stipe*** 45–80 × 7–12 mm, cylindric, at times slightly widened towards the base, this latter often subbulbous, surface faintly striate in the area below point of lamellae attachment, appressed squamulose at half or two thirds of its inferior length, glabrescent upwards, whitish or yellowish-white, the area above and below the ring pinkish-brown to brownish-orange (5AB4); squamules brownish (5AB5); initially solid, with age becoming stuffed or fistulose; base with white, somewhat coarse rhizomorphs. ***Veil*** pendant, superior, membranous, double, whitish, more or less cottony-arachnoid below, persistent or partially persistent, ***Annulus*** attached or semi-moveable, persistent, thick, with coarse, flattened, brown or brownish, cremallation-like protuberances. ***Context*** whitish, unchanging, with yellow-brown stains at stipe base, hygrophanous, fleshy-fibrous. ***Odor*** fruity. ***Smell*** mild.

Basidiospores 5–8.5 × 2.5–3 µm; $\bar{x}$ = 7.1–7.7 × 2.7–2.8 µm; ***Q*** = 2.7–2.8 [in the *lectotype* 6–8 (–8.5) × (2–) 2.5–3 (–3.5) µm, $\bar{x}$ = 7.3 × 2.8 µm, ***Q*** = 2.7], cylindric, somewhat basally truncate, with a faint suprahilar depression, attenuated and slightly curved towards the apex, hyaline to pale hyaline-greenish in KOH, yellowish in mass, at times with dense and weakly refractive contents, smooth, thin to slightly thick walled (up to 0.5 µm thick), dextrinoid or not dextrinoid. ***Basidia*** 13–16 × 5–6 µm, bi- or tetrasporic, clavate, thin walled, hyaline. ***Pleurocystidia*** absent. ***Cheilocystidia*** 14– 48 × 9–26 µm (28-63 × 7-17.5 µm in the *lectotype*), clavate to broadly clavate or broadly utriform, at times pedicellate, apically rounded and some appearing capitate, other at times nodulose, occasionally mucronate, projecting beyond the hymenial layer, hyaline to yellowish, frequently with dense, at times granulose, yellowish-brown contents (often appearing

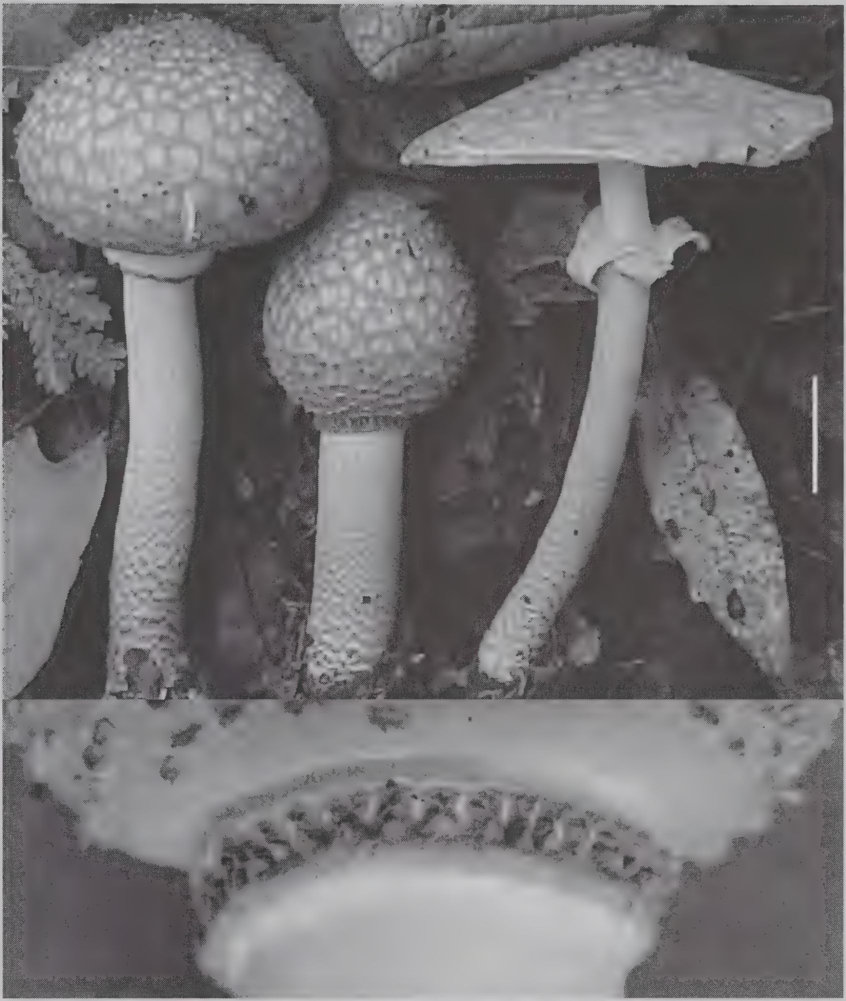


Fig. 5. ***Lepiota hemisclera.*** Basidiomes and close up (below) of the annullus (*Bandala 3626*). Bar = 20 mm.

as a circular or irregular apically located vacuole), with some floating, refringent, crystal-like elements, wall up to 1 μm thick, numerous, clamped; apex at times with mucilaginous remnants as a hyaline incrustation. ***Pileipellis*** a loose trichoderm of approx. 450 μm thick, consisting of interwoven hyaline, hyphae 3–5 μm broad, thick-walled (0.5–1 μm thick), yellow-brown to yellow-green, clamped, at times there are some intercalar rows of subisodiametric, clavate or subcylindric clamped elements, which are similar to those forming the scales; pyramidal scales built of chains of versiform elements (subisodiametric, ventricose, pyriform, subellipsoid, mucronate), 10–63 × 7–13 μm, clamped, thick-walled (1–1.5 μm), yellow or yellowish-brown, the wall clearly

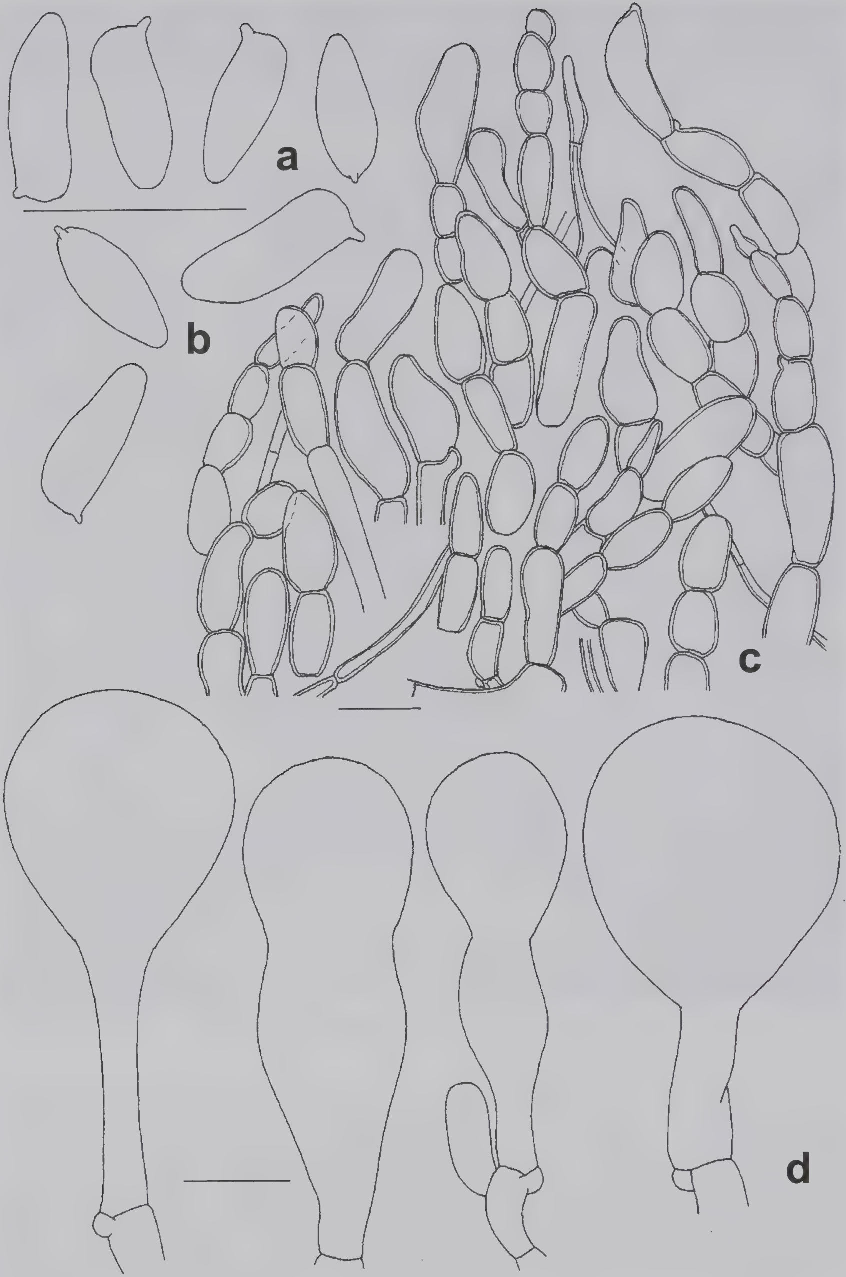


Fig. 6. ***Lepiota hemisclera.*** a-b: basidiospores; c: elements of the scales on pileus; d: cheilocystidia (a, c: *Bandala 3626;* b, d: *Bandala 3769*). Bar a-b, d = 10 µm, c = 25 µm.

appears more yellow than the contents or almost yellowish-green. ***Context*** hyphae 2–10 μm diam., thin walled; with abundant sphaerocyte-like elements towards the hymenial region, these of 15–40 μm diam. ***Hymenophoral trama*** more or less regular, hyphae 2–16 μm diam., with sphaerocyte-like elements towards the connection with the context. ***Veil*** built by cylindric hyphae 2–12 μm diam., thin or thick-walled (0.5–1 μm thick), more or less hyaline, with a slight greenish tint, similar to that of the pileipellis elements. ***Stipe context*** with yellowish, somewhat parallel hyphae 4–16 μm diam., thin or somewhat thick-walled (< 1 μm thick), clamped. ***Stipitipellis*** a loosely arranged tissue, composed of hyphae 3–4 μm thick, septate, curled, branched, clamped, at times ending in a more or less clavate element of 6–7 μm diam. at apex. ***Clamp connections*** present.

Habitat. Gregarious, on soil, among leaf debris, at times among dead leaves and *Selaginella,* near *Platanus,* at forest edge, at 1300 m.

> **SPECIMENS EXAMINED** — MEXICO. VERACRUZ: Xalapa Co., km 2.5 old road Xalapa-Coatepec, Instituto de Ecología, Botanical Garden Francisco J. Clavijero, native wooded area, 3.VII. 2002, *Montoya 4008, Bandala 3626;* 30.VI. 2003, *Bandala 3768, 3769* (all at XAL).
>
> **Other material studied** — CUBA. On logs, July 27 (year not mentioned), *J. Wright 120* (*Lectotype* of *Agaricus hemisclerus,* K).

Remarks. This *Lepiota* species macroscopically can be recognized by the entirely reticulate aspect of the pileus surface bearing pyramidal detersile scales, the persistent thick, superior, cog-wheel-like annulus, pendant veil, and the appressed squamulose inferior half of the stipe surface. These characteristics in combination with the cylindric basidiospores and both swollen cheilocystidia and elements of pileipellis, taxonomically distinguish *L. hemisclera.*

The diagnosis of this taxon is complemented with the above macro- and microscopic data recorded on fresh collections gathered in Xalapa, as well as those observed after re-examination of the lectotype of *Agaricus hemisclerus.* Two specimens collected in Cuba by Ch. Wright (no's *57* and *120*) were cited in the original description (Berkeley and Curtis 1868), one of them (*120*) selected later by Pegler (1987) as the lectotype. The Mexican specimens are indistinguishable from specimen *Wright 120,* this latter however, presents somewhat collapsed lamellae edges making difficult the study of cheilocystidia, which more or less recover when mounted in KOH (cf. figs. 6-8). It is also remarkable that the dextrinoid reaction of basidiospores is not always consistent in both the lectotype and Mexican samples. In the brief original description, based on the data recorded in fresh collections by Ch. Wright, Berkeley and Curtis (1868) described the annulus as "...amplo reflexo..." and with regard to the pileus surface they recorded it as "... sometimes prettily clouded...". The pileus of the lectotype, like that of Mexican collections, is certainly reticulate, the dry sample still exhibits a faint, wide mesh produced by paler and darker areas (it also presents scattered, pyramidal to echinate, detersile dark scales). The stipe bears a well preserved thick annulus (this holding a membranous-cottony almost pendant veil) which on its outer side presents brown, coarse, teeth-like protuberances. It should be pointed out that these two striking macroscopic features unique for *L. hemisclera* were not mentioned in subsequent cites after diagnosis of the species (Morgan 1906; Murrill 1911, 1914) including a more

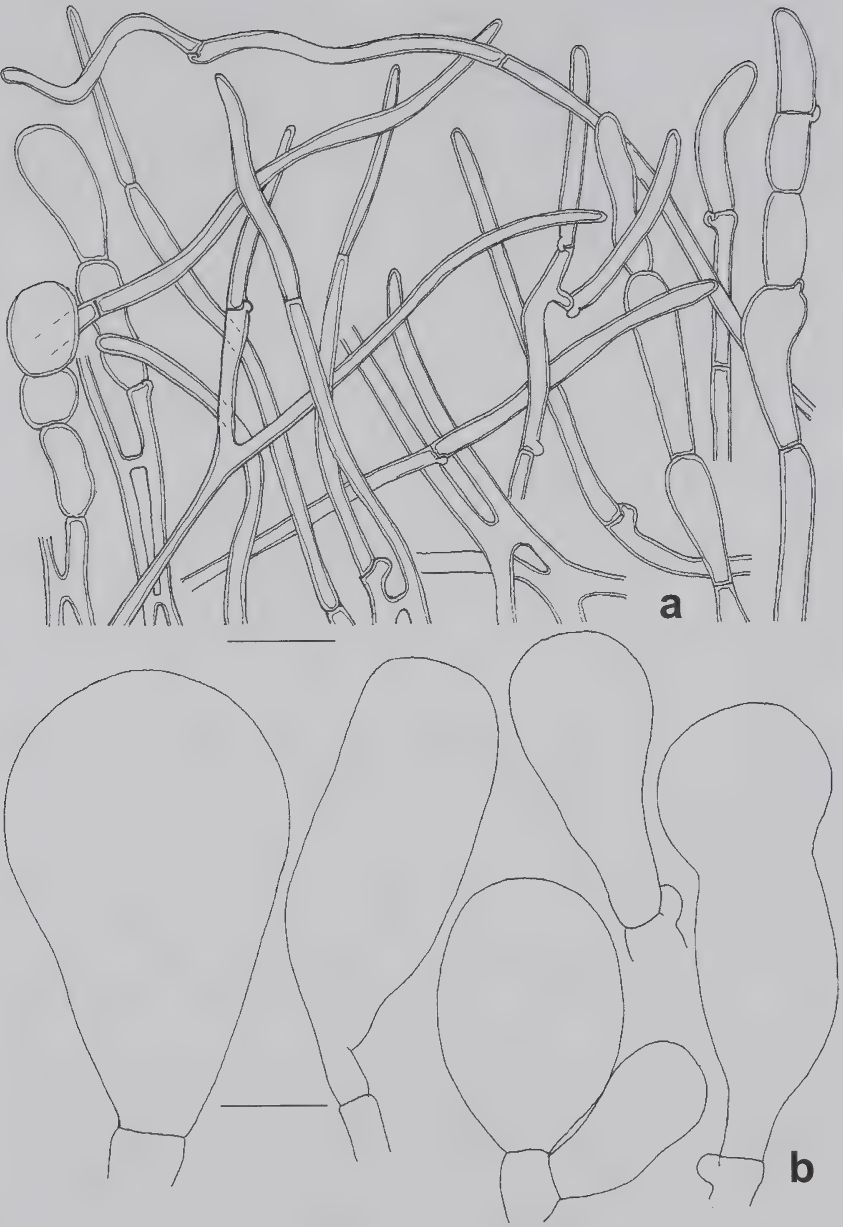


Fig. 7. ***Lepiota hemisclera.*** a: pileipellis elements; b: cheilocystidia (a: *Bandala 3626;* b: *Bandala (3769*). Bar a = 20 μm, b = 10 μm.

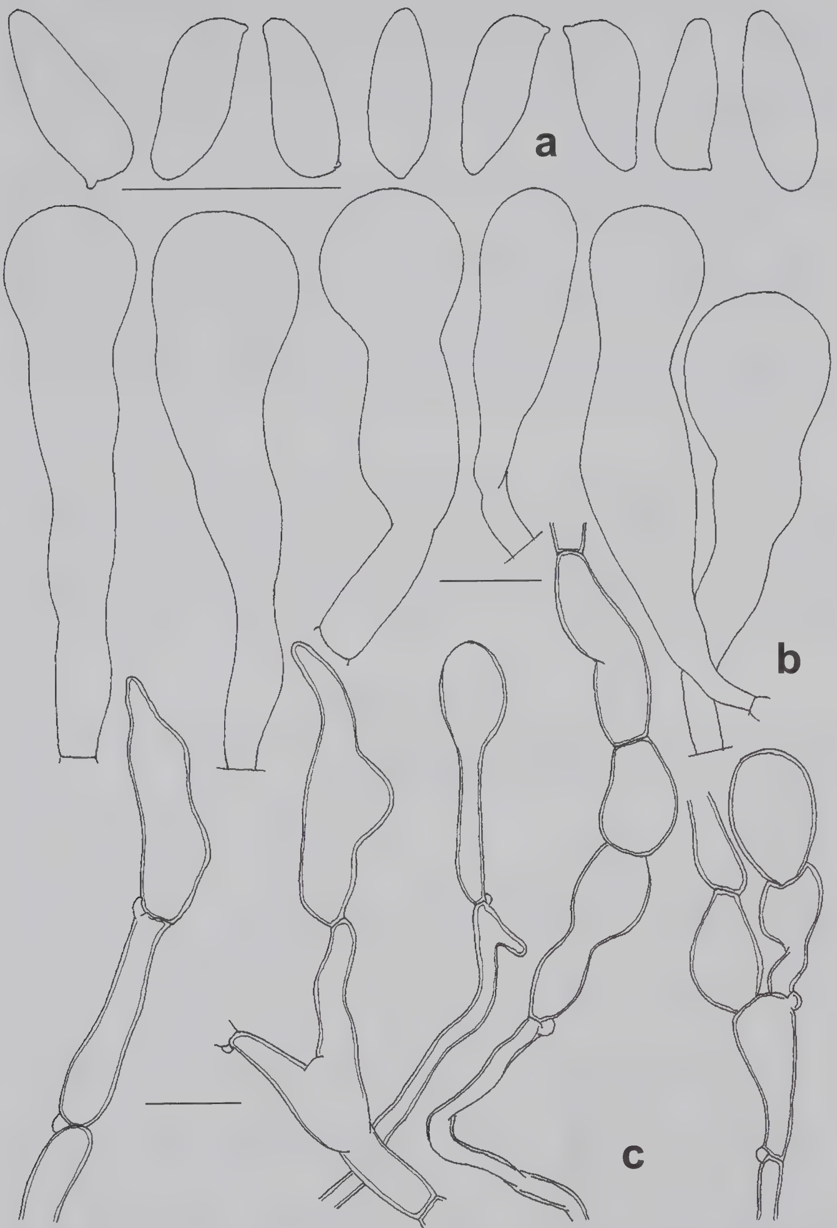


Fig. 8. ***Lepiota hemisclera.*** a: basidiospores; b: cheilocystidia; c: elements of the scales on the pileus (*Wright 120*). Bar a-b = 10 µm, c = 20 µm.

modern re-description (macro- and microscopic) of the taxon provided by Pegler (1983 as *Cystolepiota,* Pegler). A species with remarkable morphological similarities is *Lepiota aspera* (Pers.: Fr.) Quél., which indeed differs macroscopically by its pileus lacking a mesh, its pendant veil directly attached to the stipe (without an annulus unlike that of *Lepiota hemisclera*), and the stipe bearing squamulose veil remnants (Horak 1980; Candusso & Lanzoni 1990).

The information on *L. hemisclera* provided by Ovrebo (1996), Mata (1999) and Halling and Mueller (2005) based on Costa Rican collections, and that by Lodge (www.cortland.edu/nsf/lodlepi.html) on Puerto Rican samples agree very well with the Mexican and Cuban (lectotype) specimens. The species is also known from Bolivia and Colombia (Halling and Mueller 2005). These data indicate that *L. hemisclera* has a noticeable distribution in the Neotropic through tropical and subtropical forests from the central area of the Gulf of Mexico to the Caribbean, Central and middle South America. In the cloud forest sites under observation near Xalapa, however, *L. hemisclera,* contrary to *L. trichroma* above discussed, is a rarely encountered agaric.

Acknowledgements

We appreciate the collaboration of curators from K and TMI herbaria in the loan of specimens and the help of Biόls. D. Jarvio and J.C. Corona during field explorations. We express our thanks to Drs. A. Batista Pereira (Universidade Luterana do Brazil) and V. Migliozzi (Gruppo Micol. Bresadola Trento-Roma) for reviewing the manuscript and providing suggestions. We appreciate suggestions made by Dr. Lorelei Norvell (Mycotaxon Editor-in-Chief) during the press process, which also helped to improve this paper.

Literature Cited

Akers BP, Angels SA, Kimbrough JW. 2000. *Leucoagaricus viridiflavoides,* a new species from Florida, with notes on related taxa. Mycotaxon 76: 39-50.

Berkeley MJ, Curtis MA. 1868. Fungi Cubenses (Hymenomycetes). Jour. Linn. Soc. Bot. 10: 280-341.

Bon M 1981. Cle mongraphique des Lepiotes D´Europe (=*Agaricaceae,* tribus *Lepiotaceae* et *Leucocoprineae*). Doc. Mycol 11: 1-77.

Candusso M, Lanzoni G. 1990. *Lepiota* s.l. Fungi Europaei 4. Saronno.

Halling R, Mueller G. 2005. Common mushrooms of the Talamanca mountains, Costa Rica. Mem. New York Bot. Gard. 90: 1-195.

Heinemann, Rammeloo J. 1985. De la mesure des spores et de son expression. Agarica 6: 366–380.

Holmgren PK, Holmgren NH, Barnett LC (eds.). 1990. Index Herbariorum. Part I. The herbaria of the world, ed. 8. New York, 693 p.

Hongo T. 1956. Notes on Japanese larger fungi (8). Journ. Jap. Bot. 31: 144-149.

Horak E. 1980. On Australasian species of *Lepiota* S.F. Gray (*Agaricales*) with spurred spores. Sydowia 33: 111-144.

Imazeki R, Hongo T. 1983. Colored illustrations of fungi of Japan vol. 1. Hoikusha Publ. 27th ed., Osaka, 181 pp.

Kornerup A, Wanscher JH. 1967. Methuen handbook of colour. 2nd ed., Methuen, London.

Mata, M. 1999. Macrohongos de Costa Rica. INBio, Santo Domingo de Heredia, 253 pp.

Morgan AP. 1906. North American species of *Lepiota*. Jour. of Mycology 12: 195-203.'

Murrill WA. 1911. The *Agaricaceae* of tropical North America II. Mycologia 3: 79-91.

Murrill WA. 1914. *Agaricaceae*. North American Flora 10: 1-76.

Munsell soil color charts. 1994. Macbeth, New York, 4 p. + 9 color charts.

Ovrebo CL. 1996. The agaric flora (*Agaricales*) of La Selva Biological Station, Costa Rica. Rev. Biol. Trop. 44 (Suppl. 4): 39-57.

Pegler DN. 1983. Agaric Flora of The Lesser Antilles. Kew Bull. Add. Ser. 9 HMSO, Kew, UK.

Pegler DN. 1987. A revision of the *Agaricales* of Cuba 1. Species descibed by Berkeley & Curtis. Kew Bull.42: 501-585.

Rzedowski J. 1978. Vegetación de México. Limusa, México, D.F.

Sathe AV, Kulkarni SM. 1980. *Agaricales* (mushrooms) of Karnatake State. Maharashtra Association for the cultivation of Science, Monograph no. 1 *Agaricales* (mushrooms) of South West India: 43-73.

Vellinga EC, Huijser H. 1993. Notulae ad Floram Agaricinam Neerlandicam XXI, *Lepiota* section *Stenosporae*. Persoonia 15: 223-240.

MYCOTAXON

Volume 94, pp. 127–132 October–December 2005

The connection of *Dothidotthia aspera* (*Botryosphaeriaceae*) to a hyphomycetous anamorphic fungus, *Thyrostroma negundinis*

ANNETTE W. RAMALEY

awramaley@yahoo.com
7 Animas Place
Durango, CO 81301 USA

Abstract—*Dothidotthia aspera* was believed to have a coelomycetous anamorph. However, cultures of the fungus from several substrata, including species of *Fendlera, Acer, Euonymus, Symphoricarpos,* and *Robinia,* were indistinguishable from cultures of the *Stigmina* hyphomycete from the same substrata. This hyphomycete, *Stigmina negundinis,* is transferred as *Thyrostroma negundinis.*

Key words—coelomycete, teleomorph

Introduction

Dothidotthia Höhn. is a member of the *Pleosporales, Botryosphaeriaceae* (Barr 1987), a family comprising genera with immersed ascomata becoming erumpent to nearly superficial on woody twigs, large-celled pseudoparenchymatous peridia, broad cellular pseudo-paraphyses, and broad, bitunicate asci. Species of *Dothidotthia* were compiled and described by Barr (1989), and a key to seven North American representatives provided. Extensive synonymy was cited with species originally described in *Amphisphaeria, Dibotryon, Didymosphaeria, Gibbera, Gibberidea, Herpotrichia, Leptosphaeria, Microthelia, Neodeightonia, Otthia, Parodiella, Plowrightia, Pseudotthia,* and *Valsaria. Dothidotthia* was known only in North America at that time. Redisposition of some species inappropriately placed in *Didymosphaeria* (Aptroot 1995) extended the range of *Dothidotthia celtidis* (Ellis & Everh.) M. E. Barr to Britain, and *D. ramulicola* (Peck) M. E. Barr to numerous hosts and many locations in both hemispheres.

Anamorphs for the *Botryosphaeriaceae* are described as coelomycetous when known (Barr 1987), and for *Dothidotthia* as associated coelomycetous *Diplodia*-like states (Barr 1989, Kirk et al 2001). Cultures of *Dothidotthia ramulicola* (Barr 1989) produced conidiomata with brown, mostly 1-septate conidia similar to those found in conidiomata resembling and intermixed with ascomata on the substratum. Ascomata on the type specimen of *D. aspera* (Ellis & Everh.) M. E. Barr are associated with conidiomata bearing brown, mostly 3-septate conidia with an occasional longitudinal septum in one cell. For the present study, specimens of *Dothidotthia* were collected from *Fendlera rupicola* A. Gray, *Symphoricarpos rotundifolius* A. Gray, *Euonymus alatus* (Thunb.) Siebold, *Robinia neomexicana* A. Gray, and *Acer negundo* L.

Following Barr's present concept of the genus, all these collections represent *D. aspera*. A few conidiomata with brown, 3-septate conidia similar to those described by Barr (1989) on the *D. aspera* type, were present on the *Symphoricarpos* collection. Interestingly, a different coelomycete with conidia having most of these traits was abundant on the *Robinia* collection, and a third coelomycete with brown, aseptate conidia was abundant on collections of *Acer negundo*. Of even greater interest, perhaps, ascomata on all collections were accompanied by a hyphomycete bearing nearly identical conidia. Ascomata on *Fendlera, Robinia, Euonymus,* and *Acer* were sometimes in the middle of hyphomycete growth. These ascomata were young and vigorous, not being overgrown. Conidia in all collections resembled those illustrated by Ellis (1971) and Hughes (1955) and others, as well as that pictured by Shoemaker (1963) in his discussion of *Griphosphaerioma kansensis* and *Gibbera andersonii,* a synonym of *D. aspera*. The hyphomycete, a species of *Stigmina s. lat.*, has large, brown, 2-septate, ellipsoid conidia born on percurrently proliferating conidiogenous cells.

Stigmina Sacc. is a genus broadly interpreted by Ellis (1959, 1963) with species having widely varying traits. To begin to resolve this situation, Sutton and Pascoe (1989) proposed two groups, each comprising species within a range of similarities. Their first group, *Stigmina sensu stricto,* includes those species that are foliicolous, associated with stomata, with both immersed and superficial mycelium, and brown, distoseptate conidia, born on percurrently proliferating conidiogenous cells, characters shared with the lectotype. The second group they propose includes those *Stigmina* species nearly exclusively restricted to stems, with pulvinate stromata, and brown, multiseptate to dictyoseptate conidia born on conidiogenous cells that proliferate percurrently. *Thyrostroma* Hőhn. was offered as a suitable genus for species with these traits presently in *Stigmina*. Hőhnel (1911) established *Thyrostroma* for *Coryneum compactum* Sacc., a species with the above characters and the brown, dictyoseptate conidia specified in the generic description. In addition, *T. kosaroffi* (Briosi) Hőhn. was removed from *Steganosporium*.

This study was undertaken to identify the anamorph of *Dothidotthia aspera* if it were present among any of the three coelomycetes in the collections from *Symphoricarpos, Acer,* and *Robinia*, or the hyphomycete present in all collections.

Materials and Methods

Anamorphs were cultured by placing conidia in a drop of sterile tap water in a Petri plate of commercial potato dextrose agar (PDA, Difco) and spreading the droplet with a sterile glass rod. Germinated conidia were transferred to PDA tubes. Conidia from the *Symphoricarpos* collection were obtained from a tiny bit of mycelium abutting an ascoma. At the time of transfer, each germling was examined with the compound microscope to be certain it originated from a conidium and not a stray ascospore. However, all the resulting cultures lacked vigor and grew very little. A second attempt to culture the hyphomycete from a later collection from the same site yielded one healthy-appearing colony from a single conidium. Teleomorphs were cultured by opening an ascoma in a drop of sterile tap water, rinsing asci and groups of asci in three consecutive droplets of sterile tap water, and isolating individual asci onto Petri plates of PDA. All cultures were maintained on PDA in tubes at room temperature (RT, 18–22 C) in incident daylight on a table top.

Results

Colonies of *Dothidotthia* and the associated hyphomycete originating from each substratum showed small differences from such isolates from any other substratum. In contrast, the ascomycete and hyphomycete isolates from any one substratum were identical. Conidium production was rare in plates or tubes, but a few conidia were found in at least one culture of *Dothidotthia* and one culture of the associated hyphomycete from each substratum. Those conidia from the cultured hyphomycete and ascomycete on a particular substratum were identical to one another. Neither colonies nor conidia in cultures of the three associated coelomycetes with brown conidia resembled colonies or conidia of *Dothidotthia* from any of the substrata.

Discussion

The slight differences among cultures from the different substrata as well as the similarity of cultures from the hyphomycete and ascomycete on each substratum strongly suggested the two are anamorph and teleomorph of the *Dothidotthia* species involved. The connection was confirmed by production of hyphomycete conidia in cultures from asci of the ascomycete from each substratum.

Four *Stigmina s. lat.* species which Sutton and Pascoe (1989) suggested could be included in *Thyrostroma* have stout, 2-septate, smooth conidia resembling those on the hyphomycete associated with all the above collections of *Dothidotthia.* These species are *S. trimera* (Sacc.) B. Sutton from *Robinia pseudoacacia*, *S. negundinis* (Berk. & M. A. Curtis) M.B. Ellis from *Acer negundo, S. hartigiana* (Sacc.) M.B. Ellis from *Acer campestre,* and *S. pallida* (Ellis & Everh.) M.B. Ellis from *Rhus, Malus, Cydonia,* and *Salix. Stigmina pallida* is based on *Exosporium pallidum* Ellis & Everh. for which *E. rhoinum* Dearn. & Barthol. is considered a later name, and cited as a synonym of *Sciniatosporium pallidum* (Ellis & Everh.) Morgan-Jones, an illegitimate name (Sutton 1972) proposed for many *Stigmina* species (Morgan-Jones 1971) including *S. pallida.* Although found on an *Acer* species, *Stigmina hartigiana* is an unlikely candidate for the *Dothidotthia* associate on *Acer negundo*. The species is not reported from the U.S. (Farr et al 1989), and though conidial size varies among collections, conidia of the *Dothidotthia* associate are broader and longer than any conidia of *S. hartigiana* (Ellis 1959). The other three species are problematic. Hughes (1955) treated these fungi as species of *Coryneum* and concluded that study of collections and cultures from the different substrata were necessary as these taxa were more readily identified by substrata than by their slight morphological differences, that they might even represent a single species.

Cultures and collections used in the present study tend to support Hughes' idea. Comparison of conidial dimensions from all the substrata used in this study, all associated with the same teleomorph, shows no clear separation from any substratum or from conidial dimensions from the literature for *Stigmina negundinis, S. trimera,* and *S. pallida* (Table 1). This lack of conidial size distinction as well as the broad distribution of *Dothidotthia aspera* suggests these all represent a single species in agreement with

Table 1. Conidial size (μm) for the *Dothidotthia aspera* associate and similar *Stigmina s.l.* species

Fungus	**Substrate**	**Reference**
Stigmina pallida		
39–48 X 16–19	*Rhus toxicodendron*	Ellis, 1976
35.5–55 X 15.5–19.5	*Rhus glabra*	Hughes, 1955
28–42 X 13–19	*Rhus glabra*	Ellis & Everhart, in Hughes, 1955
Stigmina trimera		
32.5–42 X 17.5–21	*Robinia pseudoacacia*	Hughes, 1955
25–45 X 15–18	*Robinia pseudoacacia*	Sydow, in Hughes, 1955
Stigmina negundinis		
27–39 X 13–18	*Acer negundo*	Hughes, 1955
25–38 X 12–18	*Acer negundo*	Ellis, 1971
26–33 X 13–16	*Acer negundo*	Dennis, in Hughes, 1955
Dothidotthia associate		
25.5–44 X 13–19	*Euonymus alatus*	Ramaley 0411
29–41 X 14.5–19	*Robinia neomexicana*	Ramaley 0412
35–47 X 16–21.5	*Fendlera rupicola*	Ramaley 0403
28–37 X 13.5–17.5	*Acer negundo*	Ramaley 0414
28–42 X 14.5–18.5	*Acer negundo*	Ramaley 0415
28–40 X 13.5–17.5	*Symphoricarpos rotundifolius*	Ramaley 0418

Hughes' observations and suggestion. The collections from *Acer negundo* necessitate the anamorph of *Dothidotthia aspera* be designated *Thyrostroma negundinis* (=*Stigmina negundinis*), the name with priority.

In Table 1, *Stigmina trimera* measurements are from *Robinia pseudoacacia*, not *R neomexicana*, the species in this study. If *D. aspera* were found on *R. pseudoacacia,* and ascus cultures were identical to those from conidial cultures of *S. trimera*, this *Stigmina* species would be synonymous with *Thyrostroma negundinis* as suggested by Hughes (1955) and supported by the information in Table 1. Similar studies with isolates from any substratum of *S. pallida* would also place that species in synonymy. *Dothidotthia aspera* is found on *Rhus* in British Columbia (Barr, personal communication) making the synonymy of *T. negundinis* and *T. pallida* even more probable. However, *T. pallida* is not included in the synonymy quoted here because more than one *Thyrostroma*-like fungus may be present on *Rhus* species making cultures necessary for convincing evidence. Kirk et al (9/25/04) list eighteen records for the genus *Thyrostroma*. Two other species are considered synonyms of names included in this listing. None of the sixteen descriptions which were obtained was identical to *T. negundinis*. Substrata were different for any known for *T. negundinis*, and in all cases, conidia were multiseptate and regularly dictyoseptate, characters specified in the generic description for *Thyrostroma*. *T. negundinis* conidia are not dictyoseptate, but the species shares the other important *Thyrostroma* traits suggested by Sutton and Pascoe (1989)—type of substratum, pulvinate stromata, large, septate, brown conidia, and percurrently proliferating conidiogenous cells.

Taxonomy

Most of the synonymy is quoted from Ellis (1959).

Thyrostroma negundinis (Berk. & M. A. Curtis) A. W. Ramaley **comb. nov.**

Basionym: Coryneum negundinis Berk. & M. A. Curtis in Berkeley, Grevillea, 2: 153. 1874.

Exosporium negundinis (Berk. & M. A. Curtis) Hőhn., Mitteil. Botan. Inst. Tech. Hochsch. Wien 6: 41. 1929.

Thyrostromella negundinis (Berk. & M. A. Curtis) Hughes, Canad. J. Bot., 31: 619. 1953.

Stigmina negundinis (Berk. & M. A. Curtis) M.B. Ellis, Mycol. Pap. 72: 44. 1959.

Sciniatosporium negundinis (Berk. & M. A. Curtis) Morgan-Jones, Canad. J. Bot. 49: 993–1009. 1971.

Helminthosporium subcuticulare Ellis & Everh., J. Mycol., 4: 114. 1888.

Coryneum negundinis Ellis & Everh., Bull. Torrey Bot. Cl. 24: 292. 1897.

Coryneum septosporioides Sacc. & P. Syd, Sylloge Fungorum 14: 1022. 1899.

Teleomorph—*Dothidotthia aspera* (Ellis & Everh.) M. E. Barr

Specimens examined: USA. CO: La Plata Co, ca 1.75 mile up Carbon Junction Trail, dead twigs of *Fendlera rupicola*, 11.V.04, AW Ramaley 0403, BPI 871821; Durango, 7 Animas Place, dead twigs of *Euonymus alatus*, 29.VI.04, AW Ramaley 0411, BPI 871820; beside Hwy 550 across from Animas View Drive, dead twigs of *Robinia neomexicana*, 6.VII.04, AW Ramaley 0412, BPI 871822; between Animas Place and Animas River, dead twigs of *Acer negundo*, 8.VII.04, AW Ramaley 0414, BPI 871819; between Animas Place and Animas River, dead twigs of *Acer negundo,* 12.VII.04, AW Ramaley 0415, BPI 871825; San Juan Co, ca 0.5 mile up Engineer Mountain Trail from turnoff at mile 52.5, Hwy 550, dead twigs of *Symphoricarpos rotundifolius.*, 24.VI.04, AW Ramaley 0410, BPI 871823; ca 0.5 mile up Engineer Mountain Trail from turnoff at mile 52.5, Hwy 550, dead twigs of *Symphoricarpos rotundifolius.*, 24.VII.04, AW Ramaley 0418, BPI 871824. Ascus cultures from 0403, 0410, 0411, 0412, and 0414 to CBS.

Acknowledgements

I thank Margaret Barr for her many contributions—sharing of her recent considerations of *Dothidotthia* species, her report of *D. aspera* on *Rhus*, as well as her helpful review of this manuscript. I also thank Keith Seifert for his input, editorial insertions, and suggestions about *Thyrostroma/Stigmina* considerations, and Phyllis Kroupa, ILL librarian extraordinaire, for tracking down elusive references.

Literature cited

Aptroot A. 1995. Redisposition of some species excluded from *Didymosphaeria* (Ascomycotina). Nova Hedwigia 60: 325–379.

Barr ME. 1987. Prodromus to Class Loculoascomycetes. Published by the author. Amherst, MA. 168 p.

Barr ME. 1989. The genus *Dothidotthia* (*Botryosphaeriaceae*) in North America. Mycotaxon 34: 517–526.

Ellis MB. 1959. *Clasterosporium* and some allied Dematiaceae-Phragmosporae. II. Mycological Papers 72: 1–75.

Ellis MB. 1963. Dematiaceous Hyphomycetes IV. Mycological Papers 87: 1–42.

Ellis MB. 1971. Dematiaceous Hyphomycetes. Kew. CAB International Mycological Institute. 608 p.

Ellis MB. 1976. More Dematiaceous Hyphomycetes. Kew. CAB International Mycological Institute. 507 p.

Farr DF, Bills GF, Chamuris GP, Rossman AY. 1989. Fungi on Plants and Plant Products in the United States. The American Phytopathological Society, 3340 Plot Kob Road, St. Paul, Minnesota 55121, USA. 1252 p.

Hőhnel F von. 1911. Fragmente zur Mykologie no 718, Űber *Thyrococcum Sirakoffii* Bubak. Sber. Akad. Wiss. Wien 120: 470–473.

Hughes SJ. 1955. Microfungi II. *Thyrostromella* Höhn. and *Thyrostromella* Syd. Canad. J. Bot. 33: 341–349.

Kirk PM, Cannon PF, David JC, Stalpers JA. 2001. Ainsworth & Bisby's Dictionary of the Fungi, 9th ed. Wallingford, UK. CABI Bioscience, CAB International. 655 p.

Kirk PM et al. – www.indexfungorum.org,- 9/25/04.

Morgan-Jones G. 1971. *Sciniatosporium* Kalchbr., and its synonyms *Marcosia* Syd., *Stigmina* Sacc., *Thyrostroma* Höhn., and *Thyrostromella* Syd., non Höhn. Canad. J. Bot. 49: 993–1009.

Shoemaker RA. 1963. Generic correlations and concepts: *Griphosphaerioma* and *Labridella*. Canad. J. Bot. 41: 1419–1423.

Sutton BC. 1972. *Sciniatosporium* Kalchbr. Trans. Br. mycol Soc. 58: 164–167.

Sutton BC, Pascoe IG. 1989. Reassessment of *Peltosoma, Stigmina* and *Batcheloromyces* and description of *Hyphothyrium* gen. nov. Mycological Research 92: 210–222.

MYCOTAXON

Volume 94, pp. 133–135 October–December 2005

A new species of *Pertusaria* from Western Australia

ALAN W. ARCHER

alanw.archer@bigpond.com
National Herbarium of New South Wales
Mrs Macquaries Road, Sydney, N.S.W. 2000, Australia

JOHN A. ELIX

John.Elix@anu.edu.au
Department of Chemistry, Faculty of Science, Australian National University
Canberra, A.C.T. 0200, Australia

Abstract—*Pertusaria subarida* from Western Australia is described as new to science. It is distinguished by asci with eight small ascospores and the presence of planaic acid and 4,5-dichlorolichexanthone as major lichen substances.

Key words—lichens, taxonomy

Introduction

The lichen genus *Pertusaria* (*Pertusariaceae*, lichenized *Ascomycota*) in Australia is reported to contain 128 species (Archer 1997) of which eight are found in Western Australia. The only depsides occurring as major compounds in the Western Australian species are 2'-*O*-methylperlatolic acid and subconfluentic acid present in *P. gibberosa* Müll. Arg., 2'-*O*-methylperlatolic acid in *P. trachyspora* A.W. Archer, and 2-*O*-methylperlatolic acid, 2,2'-di-*O*-methylstenosporic acid, glomelliferic acid or confluentic acid in *P. trimera* (Müll. Arg.) A.W. Archer. Our examination of recent collections from Western Australia led to the discovery of an un-named species of *Pertusaria* containing the depside, planaic acid as a major compound.

Planaic acid is uncommon in the genus *Pertusaria*. Hanko (1983) did not find this compound in any European species and Dibben (1980) only reported planaic acid as a trace accessory present with other C_5-orcinol depsides in three taxa, viz: *Pertusaria neoscotica* I. M. Lamb, *P. paratuberculifera* Dibben and possibly *P. sinusmexicani* Dibben.

Materials and Methods

The specimens studied are deposited in PERTH and CANB. The morphology of the lichen specimens were examined using a Zeiss Stemi 2000C stereo microscope, and a Zeiss Axiolab compound microscope. Chemical constituents were identified by thin layer chromatography (Culberson 1972; Culberson *et al.* 1981; Culberson & Johnson 1982; Elix & Ernst-Russell 1993), high performance liquid chromatography (Elix *et al.* 2003) and comparison with authentic samples.

Taxonomic Description

Pertusaria subarida A.W.Archer & Elix, **sp. nov.**

Similis Pertusaria planaica *A. W. Archer sed ascosporis minoribus differt.*

Etymology: The specific epithet derives from the subarid habitats occupied by this species.

Type: **AUSTRALIA**. Western Australia, Wotto Nature Reserve, First North Road, 21 km by road NE of Eneabba, 29°42'29"S, 115°24'37"E, 275 m, *Eucalyptus* woodland with *Melaleuca* and laterite rocks, on *Melaleuca*, *J. A. Elix 28871*, 5 May 2004; holo: PERTH, iso: CANB.

KEY CHARACTERS — **Thallus** corticolous, off-white to pale fawn, surface smooth and dull, somewhat rimose, lacking isidia and soredia. **Apothecia** verruciform, conspicuous, scattered, sometimes confluent, flattened hemispherical, sometimes constricted at the base, 0.7-1 mm diam. **Ostioles** inconspicuous, black, punctiform, 1 to 4 per verruca. **Ascospores** 8 per ascus, hyaline, smooth-walled, 1-seriate, 40-65 μm long, 18-32 μm wide. **Chemistry** — planaic acid (major), 4,5-dichlorolichexanthone acid (minor), ±2,4,5-trichlorolichexanthone (trace), ±4-chlorolichexanthone (trace), ±4,5-dichloro-6-*O*-methylnorlichexanthone (trace), ±2'-*O*-methylperlatolic acid (trace), ±2-*O*-methylperlatolic acid (trace).

DISTRIBUTION — At present this species is known from three localities in subarid areas of south-western Western Australia.

ECOLOGY — This species is corticolous on *Melaleuca* and *Acacia*. Common associated species include *Flavoparmelia rutidota* (Hook. f. & Taylor) Hale, *Haematomma eremaeum* R. W. Rogers, *Hafellia tetrapla* (Nyl.) Pusswald, *Imshaugia subarida* (Elix) Elix, *Parmeliopsis macrospora* (Elix & J. Johnst.) Elix, *Pertusaria gibberosa*, *P. trimera*, *Physcia jackii* Moberg, *Ramalina inflata* subsp. *australis* G. N. Stevens, *Ramboldia brunneocarpa* Kantvilas & Elix and *Usnea scabrida* Taylor subsp. *scabrida*.

COMMENTS — *Pertusaria subarida* is a rare species characterized by asci with 8 relatively small ascospores and the presence of 4,5-dichlorolichexanthone and planaic acid as the major lichen substances. Planaic acid occurs as a major compound together with 4,5-dichlorolichexanthone in three other *Pertusaria* species, *P. leucothelia* Müll. Arg. (Müller 1895), *P. planaica* A.W. Archer & Elix (Archer & Elix 1992) and the recently described *P. siamensis* Jariangprasert (Jariangprasert & Anusarnsunthorn 2005). However, *P. leucothelia* is characterized by the presence of 4 rough-walled ascospores per ascus and is thus quite distinct from *P. subarida*. The new species is distinguished from *P. planaica* and *P. siamensis* by its smaller ascospores; *P. planaica* has 8 smooth ascospores per ascus, 80-100 μm long, and *P. siamensis* has 2-3 ascospores per ascus, 90-174 μm long.

SPECIMENS EXAMINED — **AUSTRALIA.** Western Australia, Gwambygine Nature Reserve, 11 km S of York, 31°58'24"S, 116°48'38"E, 245 m, *Acacia acuminata* woodland with scattered *Melaleuca* on river flats, on twigs of *Melaleuca*, *J. A. Elix 31754*, 22 April 2004 (CANB); Western Flora camp area, 20 km N of Eneabba, 29°37'30"S, 115°13'30"E, on branch of *Acacia*, *E. McCrum WF257*, 4-6 June 2005 (CANB, PERTH).

Acknowledgements

We thank the Western Australian Department of Conservation and Land Management for permission to collect lichens in Western Australia and our peer reviewers, Dr G. Kantvilas and Dr Patrick M. McCarthy, for helpful amendments to the draft manuscript.

Literature Cited

Archer AW. 1997. The lichen genus *Pertusaria* in Australia. Bibliotheca Lichenologica 69: 1-249.

Archer AW, Elix JA. 1992. Further new species and new reports of Pertusaria (lichenised Ascomycotina) from Australia. Mycotaxon 45: 417-431.

Culberson CF. 1972. Improved conditions and new data for the identification of lichen products by a standardized thin-layer chromatographic method. Journal of Chromatography 72: 113-125.

Culberson CF, Culberson WL, Johnson A. 1981. A standardized TLC analysis of β-orcinol depsidones. Bryologist 84: 16-29.

Culberson CF, Johnson A. 1982. Substitution of methyl *tert.*-butyl ether for diethyl ether in the standardized thin-layer chromatographic method for lichen products. Journal of Chromatography 238: 483-487.

Dibben MJ. 1980. The chemosystematics of the lichen genus *Pertusaria* in North America north of Mexico. Milwaukee Public Museum, Publications in Biology and Geology, number 5: 1-162.

Elix JA, Ernst-Russell KD. 1993. A Catalogue of Standardized Thin Layer Chromatographic Data and Biosynthetic relationships for Lichen Substances (2nd Edition). Australian National University, Canberra.

Elix JA, Giralt M, Wardlaw JH. 2003. New chloro-depsides from the lichen *Dimelaena radiata.* Bibliotheca Lichenologica 86: 1-7.

Hanko B. 1983. Die Chemotypen der Flechtengattung *Pertusaria* in Europa. Bibliotheca Lichenologica 19: 1-296.

Jariangprasert S, Anusarnsunthorn V. 2005. Additional new taxa in the lichen genus *Pertusaria* (Lichenised Ascomycota) from Thailand. Mycotaxon 91: 279-292.

Müller J. 1895. Lecanoreae et Lecideeae Australienses novae. Bulletin de l'Herbier Boissier 3: 632-642.

MYCOTAXON

Volume 94, pp. 137–147 October–December 2005

Molecular evidence for the taxonomic status of *Metarhizium taii* and its teleomorph, *Cordyceps taii* (*Hypocreales, Clavicipitaceae*)

Bo Huang[1], Chunru Li[1], Richard A. Humber[2],
Kathie T. Hodge[3], Meizhen Fan[1] & Zengzhi Li[*1]

zzli@ahau.edu.cn
[1] Anhui Provincial Key Laboratory for Microbial Pest Control,
Anhui Agricultural University, Hefei 230036, China

[2] USDA Plant, Soil & Nutrition Laboratory
Tower Road, Ithaca, NY 14853, USA

[3] Department of Plant Pathology, Cornell University
Ithaca, NY 14853, USA

Abstract—ITS1-5.8S-ITS2 rDNA was amplified, cloned and sequenced from the stroma of *Cordyceps taii*, mycelium of *Metarhizium taii* and cultures of *M. anisopliae* var. *anisopliae* from China. Analysis of integrated data of different strains and varieties of *M. anisopliae* and *M. flavoviride* from GenBank showed that *Metarhizium taii* should be treated as a synonym of *M. anisopliae* var. *anisopliae*. The ITS1-5.8S-ITS2 rDNA sequences of *C. taii* and *M. taii* are identical, indicating that the teleomorph of *M. anisopliae* var. *anisopliae* is *Cordyceps taii*.

Key words—molecular systematics, anamorph-teleomorph connection, synonymy *Cordyceps brittlebankisoides*

Introduction

Concern about adverse effects of chemical insecticides in the environment and the growing problem of insecticide resistance has led to considerable interest in the use of biological agents for control of insect pests (Leal et al. 1997). Many species of entomogenous fungi play important roles in pest management in many countries. *Metarhizium anisopliae* var. *anisopliae* (abbreviation: *M.a. anisopliae*) is one of the most important entomogenous fungi, and has been used to control pests in America, Brazil, Australia and China, with distinct economic and social benefits.

Green muscardine disease, one of the most common fungal diseases of insects, is caused by several taxa in the clavicipitaceous anamorphic genus *Metarhizium*. Since this genus was established, several new species or varieties have been described based on seemingly minor morphological differences from *M. anisopliae*, the type of the genus. These similarities have led some to believe that the taxonomy of this genus was

---

[*] Author for correspondence

badly confused (Milner et al. 1994). Rombach et al. (1986) recognized three species: *M. anisopliae* (Metschn.) Sorokin, *M. flavoviride* W. Gams & Rozsypal, and *M. album* Petch. Tulloch (1976) segregated the type species of the genus into two varieties, *M. a. anisopliae* and *M. anisopliae* var. *majus* (J.R. Johnst.) M.C. Tulloch (abbreviation: *M.a. majus*) primarily based on conidial length, and she accepted *M. flavoviride*. Rombach et al. (1986) segregated *M. flavoviride* into the varieties *M. flavoviride* var. *flavoviride* (abbreviation: *M.f. flovoviride*) and, by virtue of its smaller conidia, *M. flavoviride* var. *minus* Rombach et al. (abbreviation: *M.f. minus*). Rath et al. (1995) suggested (but did not validly publish) the 'variety' *M. anisopliae* var. *frigidum* based on the ability of conidia to germinate at low temperatures and on patterns of hydrocarbon utilization. The isolates assigned to this variety were found by Driver et al. (2000) to be taxonomically heterogeneous and were characterized based on ITS sequences as belonging in two clades of *M. flavoviride*. Guo et al. (1986) described three new species, *M. pingshaense* Q.T. Chen & H.L. Guo, *M. guizhouense* Q.T. Chen & H.L. Guo and *M. cylindrosporae* Q.T. Chen & H.L. Guo. *Metarhizium biformisporae* A.Y. Liu et al. (1989) should be treated as a heterotypic (facultative) synonym of *M. cylindrosporae*. Liu et al. (1989) described two types of conidia being produced by *M. cylindrosporae* – an ability not noticed by Guo et al. (1986) – and it appears that the description of *M. biformisporae* is best treated as an amplified description of *M. cylindrosporae*. In any event, the latter species was transferred into the genus *Nomuraea*, another clavicipitaceous anamorphic genus, by Tzean et al. (1993). Liang et al. (1991) named *M. taii* isolated from the new species, *Cordyceps taii* Z.Q. Liang & A.Y. Liu, and postulated a connection between *C. taii* and *M. taii* based on microcyclic conidiation. Liu et al. (2001) collected a single specimen and described it as a new species, *Cordyceps brittlebankisoides* Zuo, Y. Liu et al., whose anamorph (confirmed by the identical ITS sequences from the teleomorphic stroma and *in vitro* culture) was identified as *M. a. majus*.

Recently developed molecular techniques, and particularly DNA sequencing, provide valuable tools for studying phylogenetic relationships and clarifying the taxonomic position of confusing species. Molecular techniques have been used by several authors to study genetic variation and taxonomy of *Metarhizium*. Liu et al. (1994) determined partial sequences for selected regions from small (18S) and large (28S) subunit rRNAs of species of *Metarhizium*. Curran et al. (1994) sequenced PCR products from ITS1-5.8S-ITS2 rDNA regions of 31 strains of *Metarhizium,* and found 19 kinds of ITS1-5.8S-ITS2 rDNA regions. Rakotonirainy et al. (1994) sequenced the D1 and D2 expansion region of 28S rDNA from 42 strains of *Metarhizium*. The above results collectively confirmed (i) the monophyly of the genus *Metarhizium*; (ii) the separation of *M. anisopliae* and *M. flavoviride*; and (iii) the need to subdivide *M. anisopliae* into several varieties or species.

Driver et al. (2000) partially resolved the taxonomy of *Metarhizium* using sequence data and RAPD patterns; ITS sequence data were recognized as the most important taxonomic criterion. Based on distinctive ITS sequences, *M. flavoviride* and *M. anisopliae* were recognized to include five and four clades, respectively. In addition to recognizing *M. album* as a distinct species, the classification of Driver et al. (2000) divided *M. anisopliae* into four varieties and *M. flavoviride* into four named varieties plus one other clade not treated as taxonomically distinct. The four species of *Metarhizium* described from China (Guo et al. 1986, Liang et al. 1991) have apparently not been deposited in readily accessible culture collections and, therefore, were not included in the molecular analyses by Driver et al. (2000).

During investigation of the *Cordyceps* resources in China, a *C. taii* specimen was collected in the Dabeishan Mountains, and its presumptive anamorph, *M. taii*, was isolated from the specimen. The relationship between *Cordyceps* and *Metarhizium* was originally determined by comparison of microcyclic conidiogenesis and ascospores (Liang et al. 1991), but this important result needed more proof. The aim of this study was to providegenetic verification of the connection between *C. taii* and *M. taii* and to clarify the taxonomic status of *M. taii* by comparison of its ITS sequences with those of other *Metarhizium* isolates already included in GenBank.

Materials and methods

Fungal isolates—A specimen of *M. taii* (HS00060501) occurring as a pathogen of a noctuid (lepidopteran) larva was collected from Huoshan County, Western Anhui, China. A culture originally identified as *M. taii* (RCEF0772) was isolated from its ascospores discharged onto glass slides. A culture of *M.a. anisopliae* (RCEF0386) was isolated from a noctuid larval cadaver collected in Yuxi County, Western Anhui, China. Those isolates that are included in this study only by means of sequence data obtained from GenBank are listed in Table 1.

DNA preparation and amplification—A portion of a stroma from the field-collected specimen and from cultured mycelia of *M. taii* (isolated from discharged ascospores) were each finely ground under liquid nitrogen, and genomic DNA was extracted by use of benzyl chloride according to the method of Zhu et al. (1994). The extracted DNA was stored in 100 μl TE buffer (10 mM Tris-HCl, pH 8.0; 1 mM EDTA) at 4°C, and DNA solutions were diluted 10-fold with TE for use in PCR reaction.

The nuclear ITS1-5.8S-ITS2 regions were amplified with the primer pair described by Mavridou & Typas (1998). The PCR reactions were performed in 50 μl volume with the following components: 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 2 mM $MgCl_2$, 0.001% gelatin, 200 μM of each dNTP, 20-100 ng genomic DNA, 12 pmole of each primer, and 2.5 units *Taq* DNA polymerase (Sangon, China). The reactions were prepared on ice in 500 μl microcentrifuge tubes, overlaid with 20 μl mineral oil, and placed in a thermal cycler (Techine, UK). Cycling parameters were programmed as following: an initial denaturation at 95°C for 3min, followed by 35 cycles of denaturation at 94° for 1 min, annealing at 54°C for 1 min and extension at 72°C for 2 min, with a final extension at 72°C for 10 min. Efficiency of amplification was monitored by running 8 μl of each product through 1.2 % agarose gels using TAE buffer and visualizing PCR products with ethidium bromide.

Amplification products were purified by use of a Wizard™ PCR Preps DNA Purification System Kit (Promega Co., France) and methods provided by the company.

DNA cloning and sequencing of amplified its region—Purified ITS PCR products were cloned into T-Vectors, which we prepared from SK plasmids. These vectors were used to transform *Escherichia coli* XL1-Blue, and the plasmid DNA was extracted using an alkaline lysis method (Sambrook et al. 1989).

The primers T7/T3 (forward and reverse) were used to sequence both strands using the dideoxy-nucleotide chain termination on an ABI 3700 automated sequencer at

Shanghai Genecore Biotechnologies Company. Sequence data of *C. taii* (HS00060501), *M. taii* (RCEF0772) and *M.a. anisopliae* (RCEF0386) have been deposited in the GenBank genome sequence database with accession numbers AF348393, AF348394 and AF348395, respectively.

Table 1. Previously deposited GenBank sequences used in this study.*

GenBank sequence	FI #	Fungal taxon	Host insect / order (or other source substrate)	Country of origin
AF134150	1027	*M.a. anisopliae*	*Oxya multidentata* / Orthopt.	Pakistan
AF135210	1029	*M.a. anisopliae*	*Schistocerca gregaria* / Orthopt.	Eritrea
AF135211	1031	*M.a. anisopliae*	*Teleogryllus commodus* / Orthopt.	Australia
AF135212	1034	*M.a. anisopliae*	*Patanga succincta* / Orthopt.	Thailand
AF135213	1045	*M.a. anisopliae*	*Dermolepida albohirtum* / Coleopt.	Australia
AF135214	1091	*M.a. anisopliae*	*Teleogryllus commodus* / Orthopt.	Australia
AF135215	1114	*M.a. anisopliae*	Soil, Maquarie Island	Australia
AF135216	1156	*M.a. anisopliae*	[immunocompromised human]	Australia
AF136375	163	*M.a. anisopliae*	*Kalotermes* sp. / Isopt.	Australia
AF136376	203	*M.a. anisopliae*	*Inopus rubriceps* / Dipt.	Australia
AF136928	208	*M.a. anisopliae*	*Phaulacridium vittatum* / Orthopt.	Australia
AF137054	114	*M.a. anisopliae*	*Antitrogus parvulus* / Coleopt.	Australia
AF137055	23	*M.a. anisopliae*	*Aenolamia albofasciata* / Hemipt.	Mexico
AF137056	700	*M.a. anisopliae*	*Costelytra zealandica* / Coleopt.	New Zealand
AF137057	328	*M.a. anisopliae*	*Inopus rubriceps* / Dipt.	Australia
AF137058	379	*M.a. anisopliae*	*Inopus rubriceps* / Dipt.	Australia
AF137059	775	*M.a. anisopliae*	Soil (near mound of *Coptotermes lacteus* / Isoptera)	Australia
AF137062	987	*M.a. acridum*	*Ornithacris cavroisi* / Orthopt.	Niger
AF137065	147	*M.a. lepidiotae*	*Lepidiota consobrina* / Coleopt.	Australia
AF137061	389	*M.a. majus*	*Oryctes rhinoceros* / Coleopt.	Indonesia
AF138270	38	*M.f. flavoviride*	*Adoryphorus couloni* / Coleopt.	Australia
AF138272	1172	*M.f. minus*	*Niliparvata lugens* / Homopt.	Philippines
AF139853	1125	*M.f. novo-zealandicum*	Soil	Australia
AF139850	72	*M.f. pemphigi*	*Pemphigus treherni* / Homopt.	UK
AF137067	MaF	*M. album*	*Nephotettix virescens* / Homopt.	Philippines
AJ309332		*Cordyceps brittlebankisoides*	Scarabaeidae / Coleoptera	China

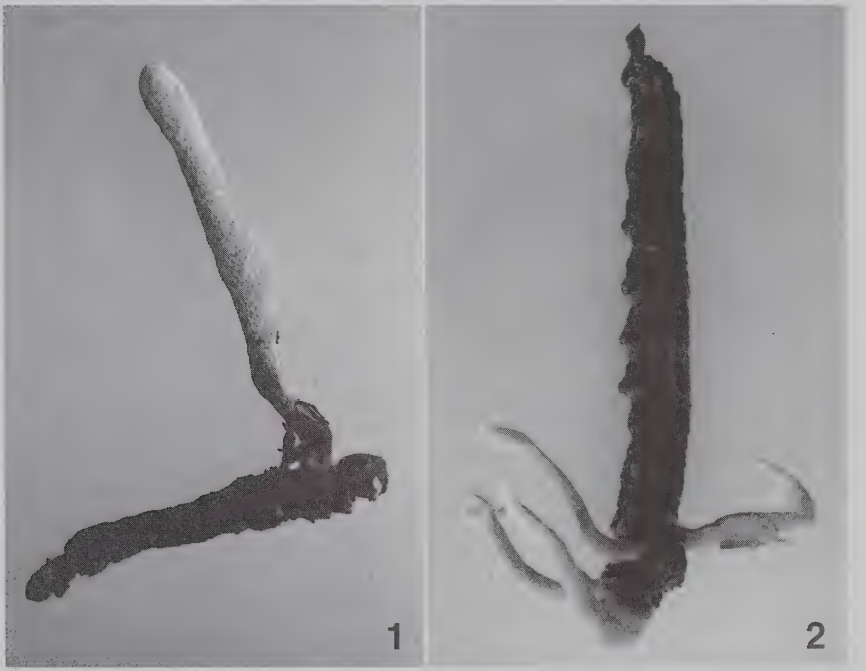
*The fungal taxonomy follows that of Driver et al. (2000). FI = CSIRO collection of entomopathogenic fungal cultures (Canberra, Australia). *M.a. = Metarhizium anisopliae*; *M.f. = Metarhizium flavoviride.* Note that FI-1029 is derived from the neotype specimen of *M. anisopliae.*

Alignments and analysis—DNA sequences generated by us and downloaded from GenBank were aligned using Clustal X 1.81 (Thompson et al. 1997), and the alignment was refined by eye. *Beauveria bassiana* (Bals.-Criv) Vuill, a related clavicipitacean anamorph, was used as an outgroup. Parsimony analysis was performed in PAUP* version b10 (Swofford 2002) using a heuristic search. A starting tree was obtained via a "closest" addition sequence, tree-bisection-reconnection was used as the branch-swapping algorithm, and MULTREES was off. Gaps were treated as missing data, and 112 ambiguously aligned characters were excluded from the analysis. Bootstrap values were calculated based on 100 replicates of a heuristic fast stepwise addition search.

Bayesian analysis was carried out using MrBayes 3.0b4 (Huelsenbeck 2000, Huelsenbeck et al 2001). We used a six parameter model to run four chains for 500,000 generations, saving a tree every 100 generations. The first 500 trees were discarded (burn in), and the remaining trees were saved to a file. A 50% majority rule consensus tree (Fig. 3) was then calculated using PAUP*.

Results

Characterization of specimen HS00060501 and its isolate, RCEF0772—The host is covered with yellow mycelium. Three stromata emerge from the head of the host, they are cylindric, 38 x 3.5–6 mm broad at stalk, with a yellowish fertile head of 20-35 x 2-5 mm. Perithecia flask-shaped with curved neck, obliquely immersed, 810-(950)-1120 x 230-380 μm. Asci cylindric, 300-490 x 3.4-5.7 μm, with ascus cap 3.1 μm in diameter. Part ascospores cylindrical, 20.0-(28.6)-34.6 x 1.4-(2.5)-3.1 μm.



Figures 1-2. *Cordyceps taii.* Lepidopteran larvae from Anhui Province, China, bearing ascigerous stromata (immature in Fig. 2) without its associated *Metarhizium* anamorph.

Colonies on Czapek's agar attaining a diameter of 35-43mm with 14 days at 25˚C, flat, with little aerial mycelium, dull gray green, reverse center deep green, subcenter to margin light sandy yellow. Cylindrical conidiogenous cells with short neck, 7.2-(14.4)-21.6 x 1-(1.4)-1.8 μm, borne in a palisade on mycelium or conidiophore. Conidia cylindric, (5.0-)7.0–9.0(-10.0) x 2.4-(2.8)-3.2 μm, two-celled conidia absent.

Based on the long part spores and other important morphological characters, the *Cordyceps* specimen (HS00060501) from Huoshan County was identified as *Cordyceps* *taii*. The morphological characters of the conidiogenous structures and conidia arising from the part spores agree with the original description of *M. taii* (Liang et al. 1991). Two more recently collected specimens of *Cordyceps taii* collected in Anhui Province are illustrated in Figures 1 and 2.

Clarification of taxonomic position of *Metarhizium taii*—Regions of the 5.8S rRNA and the complete internal transcribed spacer (ITS) regions from *M. taii* (GenBank AF348394) and *M.a. anisopliae* from China were sequenced; their total sizes were 449 and 451 nucleotides, respectively. These sequences and ITS sequences obtained from GenBank for 20 strains and 4 varieties of *M. anisopliae* were subjected to phylogenetic analysis (Fig. 3). The aligned sequences of the new Chinese isolates of *M. taii* and *M. anisopliae* showed a high degree of similarity with those of the 17 strains of *M. a. anisopliae* from Driver et al. (2000). There are 22 mutations in total, half of which were transitions (7 C/T and 4 A/G); the remaining mutations included three C/A, two T/G, one A/T as well as five putative insertions or deletions. The nature of these mutations suggests that these sequences may have diverged only recently. Among these mutations, 11 occurred in ITS1 and 11 in ITS2; the sequences of the 5.8S rRNA gene among these 18 isolates of *M. a. anisopliae* (a set of sequences including that from FI-1029, which was derived from the IMI *ex neotype* culture for *M. anisopliae*) and that of *M. taii* were identical. Divergence in 5.8S rRNA gene sequences between *M. taii* and other *Metarhizium* species or varieties varies from 0–1.8% (Tab. 2).

The parsimony search found 2864800 equally parsimonious trees, each of 376 steps and with CI=0.811 and RI=0.808. Of the 683 included characters, 465 were constant, and 106 were parsimony-informative. The strict consensus tree is shown in Fig. 3. The tree resulting from the Bayesian analysis has an identical topology.

The large number of trees obtained via the parsimony approach reflects a lack of variation among isolates of a single variety. In general, the ITS locus provided little resolution at the varietal level, but did provide support for the recognition of all but one of the varieties (*M.a. majus*). The Bayesian analysis generally agreed with the parsimony consensus tree in that each of the varieties described by Driver et al. (2000) was supported by tree topology except for *M.a. majus*. *Metarhizium taii* groups with all strains of *M.a. anisopliae* with 99% bootstrap support; if the 85th character of the alignment is excluded, then strain FI379 is included in this clade with 99% bootstrap support. Furthermore, all varieties of *M. anisopliae* recognized by Driver et al. (2000)–*M.a. anisopliae*, *M.a. majus*, *M.a. lepidiotae* Driver & Milner, and *M.a. acridum* Driver & Milner–cluster together in a clade with 100% bootstrap support and differ distinctly from *M. album* and from all described varieties of *M. flavoviride*. These results suggest that *M. taii* should properly be treated either as a variety of *M. anisopliae* or as being taxonomically indistinguishable from *M.a. anisopliae.*

Table 2 shows that the range of variation in nucleotide divergence among *M. taii* and 18 isolates of *M.a. anisopliae* from ITS1, ITS2 and both combined falls within the scope of variation among *M.a. anisopliae* isolates, and is distinctly greater than that among *M. taii*, *M.a. lepidiotae* and *M.a. acridum*. The range of variation among *M. taii* and 18 isolates of *M.a. anisopliae* from ITS1+ITS2 regions is a little less than among *M.a. majus* and 18 isolates of *M.a. anisopliae.*

Table 2. Nucleotide divergence within and among species for isolates of *M. anisopliae* [*M.a.*] *anisopliae, M.a. lepidiotae, M.a. acridum, M.a. majus* and *M. taii.**

Isolates being compared	Nucleotide divergence (%)			
	5.8S	ITS1	ITS2	ITS1+ITS2
M. taii, 18 isolates of *M.a. anisopliae*	0	0.7-3.0	1.1-2.8	1.0-2.3
M.a. anisopliae isolates	0	0-4.4	0-2.8	0.3-2.6
M. taii, FI-1029 (*ex type* of *M.a. anisopliae*)	0	1.6	2.3	2.0
M. taii, M.a. lepidiotae, M.a. acridum	0-0.6	9.6-20.5	9.1-10.6	9.3-15.1
M. taii, M.a. majus	0	3.0	1.7	2.2
M.a. majus, 18 isolates of *M.a. anisopliae*	0	2.2-4.4	0.6-2.3	1.3-2.5

*Genetic divergence (%) = (number of substitutions/total base pairs) x 100.

Relationships between *C. taii* and *M. anisopliae* var. *anisopliae* (RCEF0772)—The total size of the ITS1-5.8S-ITS2 region (449 bp) for *C. taii* (GenBank AF348393) was identical to that of *M.a. anisopliae* (RCEF0772). The sizes of ITS1, 5.8S and ITS2 in the ITS1-5.8S-ITS2 region are 134 bp, 158 bp, 167 bp, respectively. The alignment showed that *C. taii* and *M.a. anisopliae* isolated from this *Cordyceps* specimen had identical (completely congruent) ITS1-5.8S-ITS2 sequences. This suggests that *C. taii* and *M.a. anisopliae* are genetically identical. We suggest that *Cordyceps taii* and *M.a anisopliae* are the teleomorphic and anamorphic phases of a single organism.

Discussion

The new varieties *M.a. acridum* and *M.a. lepidiotum* Driver & Milner were recognized on the basis of their distinctive ITS sequence data (Driver et al. 2000). The ITS1 and ITS2 sequence data were the only characters included in their Latin diagnoses; therefore, until morphological or other characters are correlated with these genotypes, the only means to identify varieties of *Metarhizium anisopliae* within the classification is with sequence data (Driver et al. 2000).

In the phylogenetic tree, *Cordyceps brittlebankisoides* falls in the *Metarhizium flavoviride* clade. However, its purported anamorph *M.a. majus* belongs to the *Metarhizium anisopliae* clade. Liu et al. (2001) concluded that *C. brittlebankisoides* is the teleomorph of *M.a. majus* based on the morphological characteristics of its phialides and conidia; they did not compare the ITS sequences of their fungus with those of other *Metarhizium* taxa. These authors also noted that the spore sizes and cultural color of their fungus were similar to those of *M.f. flavoviride* (Liu et al. 2001). Based on the overall genetic evidence, we believe that *C. brittlebankisoides* is not the teleomorph of *M.a. majus*, but may instead be the teleomorph of a still undescribed variety of *M. flavoviride*. Such a genetically-based reclassification within *Metarhizium* echoes the genetically-based decision to transfer grasshopper- and locust-pathogenic isolates originally identified on morphological and cultural bases as *M. flavoviride* to a new variety in a different species as *M.a. acridum* (Driver et al. 2000).

The taxonomic status of *M. taii* as a species has remained both uncertain and unverified since its description. Because the nucleotide divergence between *M. taii* and the *ex type* isolate for *M. anisopliae* falls within the range of variation among *M.a. anisopliae* isolates, and very little divergence was found in the ITS1 and ITS2 sequences between *M. taii* and 18 isolates of *M.a. anisopliae*, we propose that *M. taii* is a synonym of *M.a. anisopliae*; there is not even enough distinctive genetic variation between *M. taii* and *M.a. anisopliae* to justify treating *M. taii* as a new variety of *M. anisopliae*. Tulloch (1976) separated the varieties of *M.a. anisopliae* and *M.a. majus* mainly by the shape and lengths of their conidia, (3.3-)5.0–8.0(-9.0) μm and (9.0-)10.0–14.0(-18.0) μm, respectively. The anamorph of *Cordyceps taii* was recognized as a new species mainly based on the dimensions of its conidia [(4.8-)7.8–9.6(-14) μm] and the (apparently very infrequent) formation of two-celled conidia (Liang et al. 1991). In this study, the conidia of our *M. taii* strain (RCEF0772) are clearly within the limits of *M.a. anisopliae* defined by Tulloch: the conidia of the strain isolated by Liang fell partly in the range of *M.a. anisopliae*. Longer than usual conidia in *M.a. anisopliae* have been described previously in strains from China with lengths of 7.5–8.0 μm (Guo et al. 1986) and in strains from Australia at 8-9 μm (Yip et al. 1992). The lengths of conidia from *M. taii* fall between those of *M.a. anisopliae* and *M.a. majus*, but in view of the high genetic similarity demonstrated here the slight difference between *M.a. anisopliae* and *M. taii*, and in conidial length between *M.a. anisopliae* and *M.a. majus*, can be regarded as differences among strains. Liang et al. (1991) found differences in the ability to form synnemata and two-celled conidia compared with other species or varieties of *Metarhizium*. Comparable morphological differences among isolates of other fungal entomopathogens have not been treated as taxonomically significant. For example, isolates of many diverse entomopathogenic species of *Paecilomyces* may or may not form synnemata, with the ability to do so varying on an isolate-by-isolate basis. Similarly, Li et al. (2001) reported on an isolate of *Beauveria bassiana* able to produce large synnemata, a behavior not generally known from other isolates of this globally distributed species. The combined molecular and morphological evidence presents compelling support for the conclusion that *M. taii* cannot be retained as an independent taxon at either the specific or varietal level and that it must be synonymized with *M.a. anisopliae*.

Some studies have characterized the range of variation in nucleotide divergence in ITS1 and ITS2 regions from different strains in the other fungal groups. *Epicoccum nigrum* and *Phoma epicoccina* are synanamorphs of the same biological species, and but the ranges of divergences in the sequences of their ITS1 and ITS2 genes have been found to be 0–2.8% and 0–2.6%, respectively (Arenal et al. 2000). As a standard for comparison, other studies have found infraspecific variability in ITS1 sequences for *Hymenoscyphus ericae*, *Trichoderma harzianum*, and *Ganoderma lucidum*, to be 1.2%–3.5%, 0–2%, and 0–4%, respectively (Egger & Sigler 1993, Grondona et al. 1997). Even higher infraspecific divergence has been found for other fungi such as *Gaeumannomyces graminis,* with 1.5–12.0% for ITS1 and 0–2.9% for ITS2 (Bryan et al. 1995); and *Colletotrichium acutatum* with 0–6% in the ITS1 (Sreenivasaprasad et al. 1994).

M.a. anisopliae is one of the most common and important species of entomopathogenic fungi. Like other such globally distributed and very important entomopathogens as *Beauveria bassiana* and *Beauveria brongniartii*, the teleomorph of this hyphomycete has remained uncertain or unknown. Schaerffenberg (1959) claimed that the teleomorph of *M. anisopliae* belonged to the order Sphaeriales (Ascomycota), but this hypothesis was never well supported either by Schaerffenberg or by any subsequent workers. Liang et al. (1991) provided the first credible evidence that the teleomorph of any *Metarhizium* species is a clavicipitaceous fungus belonging in the genus *Cordyceps*;

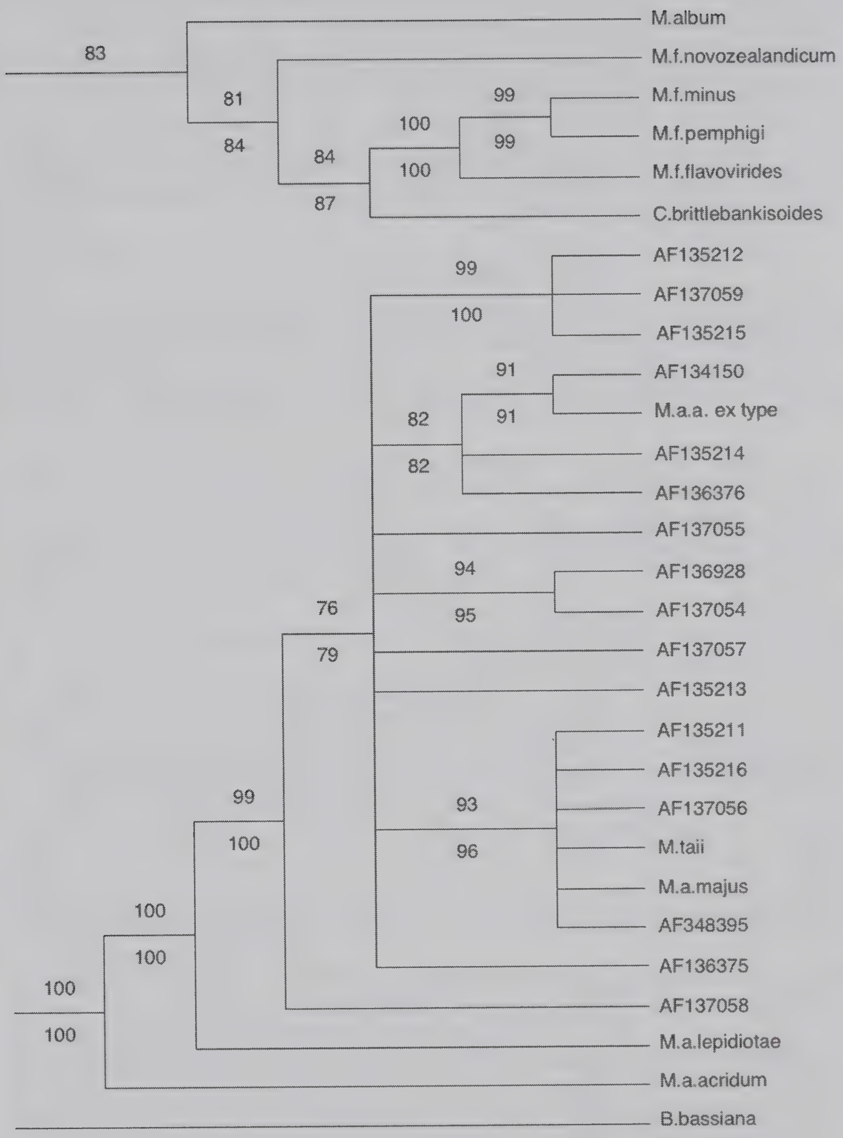

M.album
M.f.novozealandicum
M.f.minus
M.f.pemphigi
M.f.flavovirides
C.brittlebankisoides
AF135212
AF137059
AF135215
AF134150
M.a.a. ex type
AF135214
AF136376
AF137055
AF136928
AF137054
AF137057
AF135213
AF135211
AF135216
AF137056
M.taii
M.a.majus
AF348395
AF136375
AF137058
M.a.lepidiotae
M.a.acridum
B.bassiana
83
81
84
84
87
100
100
99
99
99
100
91
91
82
82
94
95
76
79
93
96
99
100
100
100
100
100

Figure 3. Strict consensus of 2864800 equally parsimonious trees based on ITS region sequence data from *Metarhizium* spp. Values above the branches indicate bootstrap support; posterior probabilities resulting from the Bayesian analysis are shown below the branches.

this connection was based on studies of secondary (microcyclic) conidiogenesis by discharged part spores. The data presented here show that *M. taii* cannot be maintained as an independent species and that the most reasonable treatment for this species is as a facultative (heterotypic) synonym of *M.a. anisopliae*:

Metarhizium anisopliae (Metschn.) Sorokīn var. *anisopliae*, [Plant Parasites of Man and Animals as Causes of Infectious Diseases] 2: 267 (1883) [in Russian]

Synonym: *Metarhizium taii* Z.Q. Liang & A.Y. Liu, Acta Mycol. Sinica 10: 260 (1991).

Because the present molecular and cultural evidence confirms unambiguously that the teleomorph of *Metarhizium taii* is *Cordyceps taii*, it must also now be recognized that the teleomorph of *M. anisopliae* var. *anisopliae* is *C. taii*.

Acknowledgments

We are grateful to Drs. Wenying Zhuang and Yijian Yao for reviewing the manuscript. This work was supported partly by the National Natural Science Foundation of China (Grant No. 30300004, 30330500), the Key Laboratory of Systematic Mycology and Lichenology (Grant No. 912.), the Anhui Provincial Science Foundation for Excellent Youth and program for "NCET".

Literature cited

Arenal F, Platas G, Monte E, Pelaez F. 2000. ITS sequencing support for *Epicoccum nigrum* and *Phoma epicoccina* being the same biological species. Mycol Res 104: 301-303.

Bryan GT, Daniels MJ, Osbourn AE. 1995. Comparison of fungi within the *Gaeumannomyces-Phialophora* complex by analysis of ribosomal DNA sequences. Appl Environ Microbiol 61: 681-689.

Curran J, Driver F, Ballard JWO, Milner RJ. 1994. Phylogeny of *Metarhizium*: analysis of ribosomal DNA sequence data. Mycol Res 98: 547-552.

Driver F, Milner RJ, Trueman WHA. 2000. Taxonomic revision of *Metarhizium* based on a phylogenetic analysis of rDNA sequence data. Mycol Res 104: 134-150.

Egger K N, Sigler L. 1993. Relatedness of the ericoid endophytes *Scytalidium vaccinii* and *Hymenoscyphus ericae* inferred from analysis of ribosomal DNA. Mycologia 85: 219-230.

Grondona I, Monte E, Garcia-Acha Y, Sutton B. 1997. *Pyrenochaeta dolichi*: an example of a confusing species. Mycol Res 101:1405-1408.

Guo HL, Ye BL, Yue YY, Chen QT, Fu CS. 1986. Three new species of *Metarhizium*. Acta Mycol Sinica 5: 177-184.

Huelsenbeck JP. 2000. MrBayes: Bayesian inferences of phylogeny (software). New York: University of Rochester.

Huelsenbeck JP, Ronquist F, Nielsen ES, Bollback JP. 2001. Bayesian inference of phylogeny and its impact on evolutionary biology. Science 294:2310-2314.

Leal SCM, Bertioli DJ, Butt TM. 1997. Amplification and restriction endonuclease digestion of the *Pr1* gene for the detection and characterization of *Metarhizium* strains. Mycol Res 101: 257-265.

Li ZZ, Li CR, Huang B, Fan MZ. 2001. Discovery and demonstration of the teleomorph of *Beauveria bassiana* (Bals.) Vuill., an important entomogenous fungus. Chinese Sci Bull 9: 751-753.

Liang ZQ, Liu AY, Liu JL. 1991. A new species of the genus *Cordyceps* and its *Metarhizium* anamorph. Acta Mycol Sinica 10: 257-262.

Liu AY, Liang ZQ, Cao L. 1989. Study on the identification of *Metarhizium biformisporae.* J Guizhou Agr Univ 2: 27-31.

Liu ZW, Guo HL, Kurtzman CP. 1994. Phylogeny of *Metarhizium* species as determined by partial ribosomal RNA sequences. Acta Mycol Sinica 13: 139-151.

Liu ZY, Liang ZQ, Whalley AJS, Yao YJ, Liu AY. 2001. *Cordyceps brittlebankisoides*, a new pathogen of grubs and its anamorph, *Metarhizium anisopliae* var. *majus*. J Invert Pathol 78: 178-182.

Mavridou A, Typas MA. 1998. Intraspecific polymorphism in *Metarhizium anisopliae* var. *anisopliae* revealed by analysis of rDNA gene species complex and mtDNA RFLPs. Mycol Res 102: 1233-1241.

Milner RJ, Driver F, Curran J, Glare TR, Prior C, Bridge PD, Zimmermann G. 1994. Recent problems with the taxonomy within the genus, *Metarhizium*, and a possible solution. Abstracts, 6th Internat. Colloq. Invertebr Pathol Microb Control, (Montpellier, France), vol. 2, pp. 109-110.

Rath AC, Carr CJ, Graham B R. 1995. Characterization of *Metarhizium anisopliae* strains by carbohydrate utilization (AP150CH). J Invert Pathol 65: 152-161.

Rakotonirainy MS, Dutertre M, Cariou ML, Brygoo Y, Riba G. 1994. Phylogenetic relationships within the genera *Metarhizium* based on 28s rRNA sequences and isozyme comparison. Mycol Res 98: 225-230.

Rombach MC, Humber RA, Roberts DW. 1986. *Metarhizium flavoviride* var. *minus* var. nov., a pathogen of plant- and leafhoppers on rice in the Philippines and Solomon Islands. Mycotaxon 27: 87-92.

Sambrook J, Fritsch EF, Maniatis T. 1989 *Molecular Cloning: A Laboratory Manual*, 2nd ed. New York; Cold Spring Harbor Laboratory Press.

Schaerffenberg, B. 1959. Zür Biologie und Ökologie des insektentötendend Pilzes *Metarrhizium anisopliae* (Metsch.) Sorok. (Entwicklung, Kultur, Lebensanspruche, Infektionsverlauf, praktische Bedeutung). Z Angew Entomol 44: 262-271.

Sreenivasaprasad S, Mills PR, Brown AE. 1994. Nucleotide sequence of the rDNA spacer 1 enables identification of isolates of *Colletotrichum* as *C. acutatum*. Mycol Res 98: 186-188.

Swofford D. 2002. PAUP*: Phylogenetic analysis using parsimony (*and other methods), version 4. Sunderland, Massachusetts: Sinauer Associates.

Tulloch M.1976. The genus *Metarhizium*. Trans Brit Mycol Soc 66: 407-411.

Thompson JD, Higgins DG, Gibson TJ. 1994. Clustal W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucl Acids Res 22: 4673-4680.

Tzean SS, Hsieh LS, Chen JL, Wu WJ. 1993. *Nomuraea cylindrospora* comb. nov. Mycologia 85: 514-519.

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG. 1997. The Clustal X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucl Acids Res 24:4876-4882.

Yip HI, Rath AC, Koen TB. 1992. Characterization of *Metarhizium anisopliae* isolates from Tasmanian pasture soil and their pathogenicity to redheaded cockchafer (Coleoptera: Scarabaeidae: *Adoryphorus couloni*). Mycol Res 96: 92-96.

Zhu H, Qu F, Zhu LH. 1994. Isolation of genomic DNAs from fungi using benzyl chloride. Acta Mycol Sinica 13: 41-47.

MYCOTAXON

Volume 94, pp. 149–154 October–December 2005

Vamsapriya indica gen. et sp. nov., a bambusicolous, synnematous fungus from India

Puja Gawas [*] & Darbhe Jayarama Bhat[**]

*pujabg@yahoo.co.in; **bhatdj@rediffmail.com
Department of Botany, Goa University
Goa-403 206, India

Abstract—*Vamsapriya indica* gen. et sp. nov. is reported from decaying culms of bamboo, *Bambusa arundinacea* (*Gramineae* or *Poaceae*), collected at Yellapur, Uttara Kannada, Karnataka, India. The fungus is unique in producing catenate, phragmosporous conidia on synnematous conidiophores with non-cicatrized, monotretic conidiogenous cells. The novel genus is described and illustrated, and compared with two closely resembling genera, *Didymobotryum* and *Podosporium*.

Key words—conidial fungi, tropical biodiversity, hyphomycetes, taxonomy

Introduction

Fungi occurring on monocot plants are fairly well documented (Ju & Rogers 1994; Fröhlich & Hyde 1999; Hyde & Alias 2000; Yanna et al. 2001). Among them, bambusicolous fungi have been well-studied (Petrini et al. 1989; Eriksson & Yue 1990; Hyde et al. 2002a) with a special interest on the pathogens (Samajpati 1984; Johnson 1985; Deka et al. 1990) and saprobes (Hyde et al. 2001; Zhou & Hyde 2002; Hyde et al. 2002b). Presently, more than 110 bambusicolous fungi have been recorded (Shenoy et al. 2005a).

During the course of studies on fungal diversity of Western Ghat forests (e.g. Pratibha et al. 2005; Shenoy et al. 2005b), we came across a unique, dematiaceous, synnematous hyphomycetous fungus on decaying culms of bamboo, *Bambusa arundinacea* (Retz.) Willd. (*Gramineae* or *Poaceae*). On careful examination, we observed a combination of morphological characters of the genera *Didymobotryum* Sacc. and *Podosporium* Schwein. Both *Didymobotryum*, lectotypified by *D. rigidum* (Berk. & Broome) Sacc. and *Podosporium* lectotypified by *P. rigidum* Schwein. are characterized by large synnemata with a stipe and apical head, branched conidiophores, monotretic conidiogenous cells and acrogenous conidia. However, the conidia of *Didymobotryum* are catenate and 1-septate whereas those of *Podosporium* are solitary and phragmosporous (Ellis 1971). The unique combination of catenate, phragmoporous conidia in a single fungus, with the other features of both *Didymobotryum* and *Podosporium* warrants placement of the bambusicolous fungus in a new species and a new genus. *Vamsapriya indica* gen. et sp. nov is described and illustrated in this paper.

Taxonomic Description

Vamsapriya Gawas & Bhat **anam. gen. nov.**

> *Ad fungos conidiales, hyphomycetes. Coloniae effusae, atro brunneae vel nigra. Mycelium substrato immersum, ex hyphis subhyalinis, septatis, ramosus, laevis. Conidiophora macronematica, synnematica, atrobrunnea, laevia, septata, ramosa, laxus apicem. Synnematica erecta, atro brunnea. Cellulae conidiogenae monotreticae, nunquan cicatricem, integratae vel discretae, terminalae, clavatae. Conidia sicca, catenulata, acrogenosa, brunnea, cylindrica, vermiformata, rotundata ad duo extremitas, phragmoseptata, angustus ad septa, acropetalibus.*
>
> *Etymology*: In Sanskrit *Vamsa*– bamboo; *priya*– loving.

Conidial fungi, hyphomycetes. *Colonies* effuse, dark brown to black. *Mycelium* immersed, composed of subhyaline, septate, branched, smooth hyphae. *Conidiophores* distinct, macronematous, synnematous, dark brown, smooth, septate, branched, wider at the apex. *Synnemata* erect, dark brown, composed of compact parallel conidiophores, fertile in the upper half. *Conidiogenous cells* monotretic, non-cicatrized, integrated or discrete, terminal, clavate. *Conidia* dry, catenate, acrogenous, brown, cylindrical, vermiform, phragmoseptate, constricted at the septa, developing in acropetal chains.

Type species: *V. indica.*

Vamsapriya indica Gawas & Bhat **sp. nov.** **Fig. 1–12**

> *Ad fungos conidiales, hyphomycetes. Coloniae effusae, atrobrunneae vel nigra. Mycelium substrato immersum, ex hyphis subhyalinis, septatis, tremes, laevis, 2.5–3.5 µm latis. Conidiophora macronematica, synnematica, atrobrunnea, laevia, septata, tremes, laxus, apicem, 3–4.5 µm lata. Synnematica erecta, rigidis, atro brunnea, 700– 870 µm longa, 80–90 µm lat. ad pessum, 28–42 µm lat. ad medius, 110–150 µm lat. ad apicem fertilis ora. Cellulae conidiogenae monotreticae, nunquam cicatricem, integratae vel discretae, terminaliae, clavatae, leviter curvatae extrinsecus, 4–12 x 2–4.5 µm. Conidia sicca, catenulata, acrogenosa, brunnea, laevia, simplicia, cylindrica, vermiformata, 2–12-septata, 10–80 x 4–6 µm, angustus ad septa, acropetalibus; conidia terminalia teres ad apicem, leviter truncata ad pessum; conidia alia leviter truncata ad duo extremitas.*
>
> Holotype: On dead and decaying bamboo twigs, Yellapur, Uttara Kannada, Karnataka, India, coll. Puja Gawas, 27/9/2005, Herb. No. IMI 393674.

Fungus hyphomycete. *Colonies* effuse, dark brown to black. Mycelium immersed, composed of subhyaline, septate, branched, smooth hyphae, 2.5–3.5 µm wide. Conidiophores macronematous, synnematous, dark brown, smooth, septate, branched, wider at the apex, 3–4.5 µm wide. Synnemata erect, rigid, dark brown, composed of compact parallel conidiophores, 700–870 µm long, up to 80–90 µm wide at the base, 28–42 µm wide in the middle, up to 110–150 µm wide at the apical fertile region. Conidiogenous cells monotretic, non-cicatrized, integrated or discrete, terminal, clavate, slightly curved towards the exterior, 4–12 × 2–4.5 µm. Conidia dry, catenate, acrogenous, brown, smooth, simple, cylindrical, vermiform, 2–12-septate, constricted at the septa, 10–80 × 4–6 µm, developing in acropetal chains; terminal conidia rounded at the apex, slightly truncate at the base; other conidia slightly truncate at both ends.

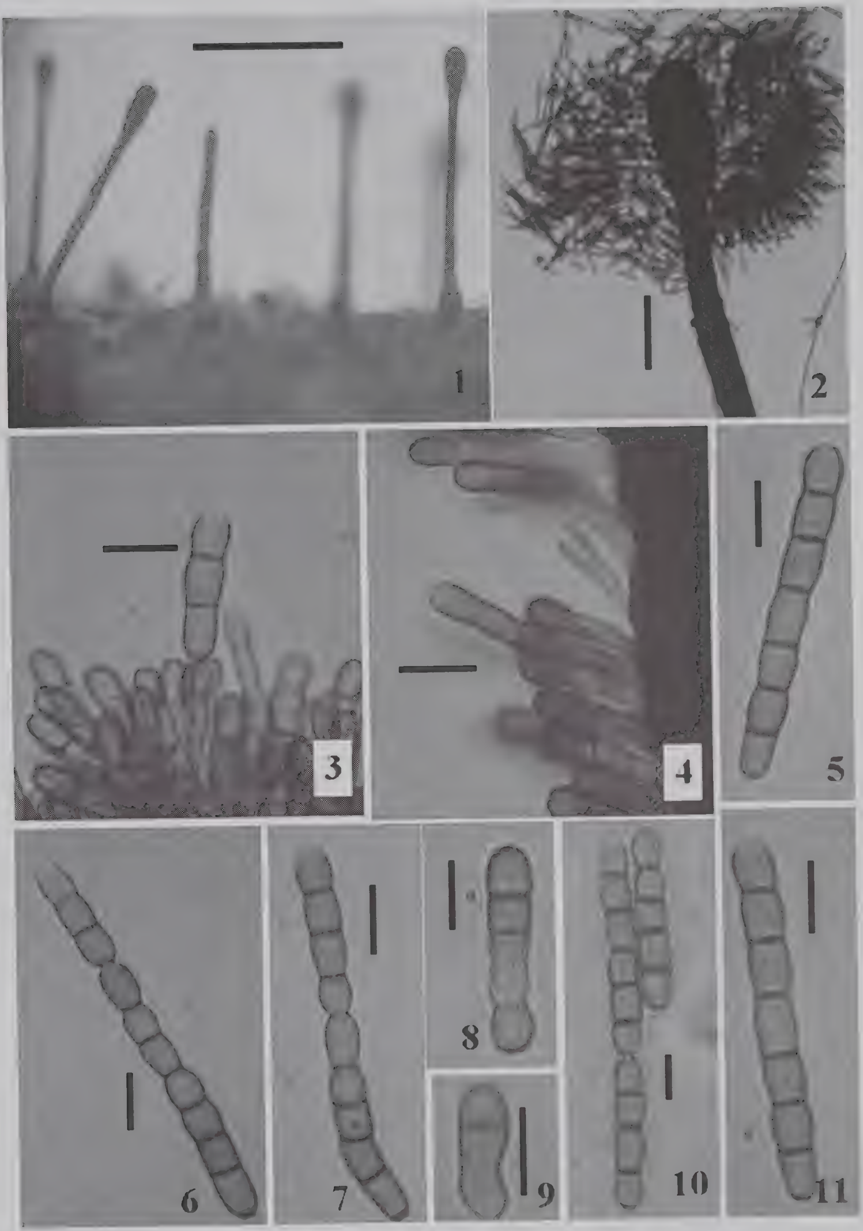


Fig. 1-11. ***Vamsapriya indica.*** 1. Stereomicroscopic image of the synnemata (bar = 0.5 cm), 2. Fertile apical zone (bar = 200 µm). 3,4. Conidiogenous cells showing monotretic conidiogenesis (bars = 10 µm). 5-11. Morphological variation in conidial size and display of catenate nature of the conidia (bars = 10 µm)

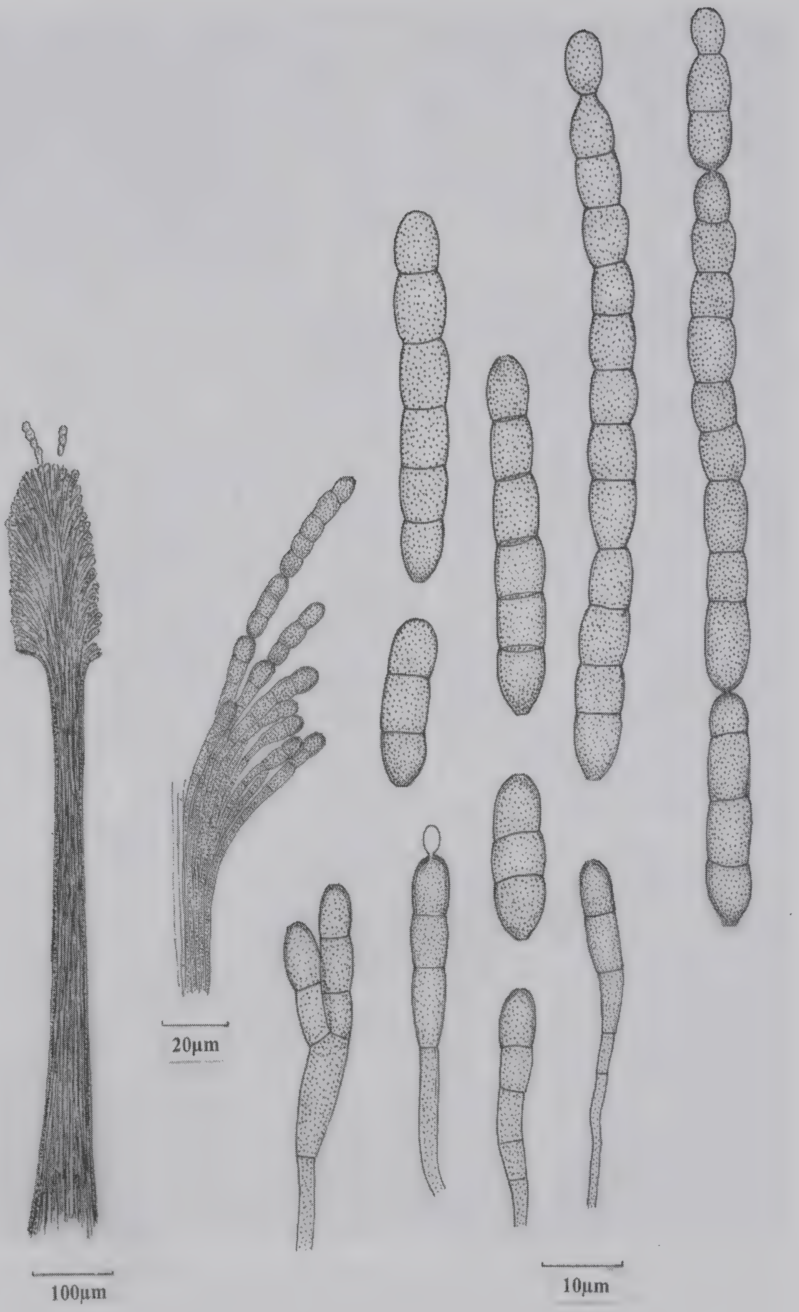

20µm
100µm
10µm

Fig. 12. ***Vamsapriya indica.*** Synnemata, conidiogenous cells and conidia

Discussion

Vamsapriya is morphologically similar to *Didymobotryum* and *Podosporium*. All three genera have brown to black rigid synnemata with compactly arranged brown conidiophores, monotretic, integrated, clavate to cylindrical conidiogenous cells bearing dry, acrogenous, simple conidia. *Didymobotryum* differs from *Podosporium* in having catenate, ellipsoidal-cylindrical, 1-septate conidia. *Podosporium*, in contrast, has solitary, obclavate, multiseptate conidia (Ellis, 1971). *Vamsapriya* exhibits a combination of morphological characters of both these genera, bearing catenate, cylindrical to vermiform, multiseptate (phragmosporous) conidia. The catenate conidia do not support disposition of *Vamsapriya* in *Podosporium* and their phragmosporous character makes it difficult to accommodate in *Didymobotryum*.

Acknowledgements

This study was supported by a Special Assistance Programme of the UGC, New Delhi, to the Department of Botany, Goa University and a research grant from the CSIR, New Delhi to DJB. PG gratefully acknowledges receipt of a Research Assistantship of the CSIR, New Delhi. We are thankful to B.D. Shenoy (The University of Hong Kong) for reading the manuscript. We are grateful to Drs. E.H.C. McKenzie (Landcare Research, Auckland, New Zealand) and B. Kendrick (University of Victoria, B.C. Canada) for kindly reviewing the manuscript.

Literature cited

Deka PC, Baruah G, Devi M. 1990. A preliminary investigation of disease of bamboo in northeast region of India. Indian Forester 116: 714–716.

Ellis MB. 1971. Dematiaceous Hyphomycetes. Commonwealth Mycological Institute, Kew, Surrey, UK.

Eriksson OE, Yue Jz. 1990. Notes on bambusicolous pyrenomycetes. Mycotaxon 38: 201–220.

Fröhlich J, Hyde KD. 1999. Biodiversity of palm fungi in the tropics: are global fungal diversity estimates realistic? Biodivers. Conserv. 8: 977–1004.

Hyde KD, Alias SA. 2000. Biodiversity and distribution of fungi associated with decomposing *Nypa fruticans*. Biodivers. Conserv. 9: 393–402.

Hyde KD, Ho WH, McKenzie EHC, Dalisay T. 2001. Saprobic fungi on bamboo culms. Fungal Divers. 7: 35–48.

Hyde KD, Zhou D, Dalisay T. 2002a. Bambusicolous fungi: A review. Fungal Divers. 9: 1–14.

Hyde KD, Zhou D, McKenzie EHC, Ho WH, Dalisay T. 2002b. Vertical distribution of saprobic fungi on bamboo culms. Fungal Divers. 11: 109–118.

Johnson GI. 1985. Rust (*Dasturella divina*) of *Bambusa* spp. in Australia. Australas. Plant Pathol. 14: 54–55.

Ju YM, Rogers JD. 1994. *Kretzschmariella culmorum* (Cooke) comb. nov. and notes on some other monocot-inhabiting xylariaceous fungi. Mycotaxon 51: 241–255.

Petrini O, Candoussau F, Petrini LE. 1989. Bambusicolous fungi collected in southwestern France 1982-1989. Mycol. Helv. 3: 263–279.

Pratibha SJ, Puja G, Shenoy BD, Hyde KD, Bhat DJ. 2005a. *Chalara indica* sp. nov. and *Sorocybe indicus* sp. nov. from India. Cryptogamie Mycol. 26: 97–103.

Samajpati N. 1984. Decay and chemical changes in the wood of *Bambusa arundinacea* caused by *Daedalea flavida*. Phytopathol. Z. 110: 203–206.

Shenoy BD, Jeewon R, Hyde KD. 2005a. *Oxydothis bambusicola*, a new ascomycete with a huge subapical ascal ring found on bamboo in Hong Kong. Nova Hedwigia 80: 511–518.

Shenoy BD, Vijaykrishna D, Cai L, Jeewon R, Bhat DJ, Hyde KD. 2005b. *Pseudohalonectria miscanthicola* sp. nov. and three interesting fungi from tropics. Cryptogamie Mycol. 26: 123–132.

Yanna, Ho WH, Hyde KD. 2001. Fungal communities on decaying palm fronds in Australia, Brunei and Hong Kong. Mycol. Res. 105: 1458–1471.

Zhou D, Hyde KD. 2002. Fungal succession on bamboo in Hong Kong. Fungal Divers. 10: 213–227.

MYCOTAXON

Volume 94, pp. 155–158 October–December 2005

Two new species of *Hypogymnia* (*Lecanorales*, Ascomycota) with pruinose lobe tips from China[*]

XINLI WEI [1,2] JIANGCHUN WEI [*,1]

weijc2004@126.com

[1]Systematic Mycology & Lichenology Laboratory
Institute of Microbiology, Academia Sinica
Zhong-guan-cun, Bei-yi-tiao#13, Beijing 100080, China

[2]Graduate School of Chinese Academy of Sciences, Beijing 100039, China

Abstract—Two new species of lichens are described from China, viz. *Hypogymnia pseudopruinosa* (in Yunnan) and *H. subfarinacea* (in Sichuan and Yunnan). They are characterized by means of morphology and secondary chemistry. Latin diagnoses, English descriptions, and habitus photographs are provided.

Keywords—*H. macrospora*, *H. farinacea*, lichen substances

Until recently, forty species of *Hypogymnia* (Nyl.) Nyl. have been reported from China (Wei 1991; Chen 1994; McCune & Obermayer 2001; McCune & Tchabanenko 2001; McCune et al. 2003). During our studies on the lichen flora of China, two new species of the genus were collected from Yunnan and Sichuan Provinces, which are described in this paper. The gross morphology and anatomy were examined using the dissecting microscope (ZEISS Stemi SV 11) and compound microscope (OPTON ⊠). The lichen substances were detected by colour reagents and thin-layer chromatography (Culberson & Kristinsson 1970; Culberson 1972; White & James 1985).

Hypogymnia pseudopruinosa X.L. Wei & J.C. Wei, sp. nov. **Plate I: A-B**

Hypogymniae macrosporae similis, sed sporis minoribus, lobi pruina obductis et atranorina deest.

Type: China. Yunnan, Dêqên county, alt. 4100 m, on dead branches of *Sabina* sp., X. Y. Wang, X. Xiao & J. J. Su 7606, 29 August 1981 (holotype, HMAS-L).

Thallus foliose, tightly appressed; with subdichotomously branched lobes of 1-2 mm wide and 5 mm long; upper surface dark brownish-yellow, partly black, with black margin, rugose, glossy, lacking isidia, soredia and lobules but with dense layer of pruina limited to the lobe tips; lower surface black, brown near the apices, rugose, glossy, and with round perforations at the lobe tips and on the lower surface.

---

[*] The project was supported by the National Natural Science Foundation of China (30499340)

Upper cortex prosoplectenchymatous, pale yellow, 12-14.5 μm thick; algal layer 10-14.5 μm thick, consisting of green, subspherical cells of 7.5-9.5 μm in diameter; hyphae in medulla colorless, about 2 μm in diameter; lower cortex prosoplectenchymatous, pale yellow, 10 μm thick.

Apothecia rare, 1-3 mm in diameter, stipitate; disc yellow brown or red brown, glossy, concave at first and then slightly plane with entire and thin margin; epithecium brown, 7-9 μm thick; hymenium colourless, 32-36 μm thick; asci clavate, 8-14 × 23.5-25 μm, 8-spored; spores simple, colourless, ellipsoid to nearly spherical, 3.5-4 × 5.5-7 μm; paraphyses linear, septate, 2 μm wide, slightly swollen at the tips; hypothecium colourless, 27-36 μm thick. Pycnidia not seen.

Chemistry. Cortex K-, C-, P-; medulla K+ yellow, C-, P+ orange yellow → orange red; containing physodalic and physodic acids, and a pale spot in R_f class 6 (solvent system C).

Comments: The new species resembles *H. macrospora* (J.D. Zhao) J.C. Wei (Zhao 1964; Wei 1991) at first sight, but differs by having a dense layer of pruina limited to the tips of the lobes and smaller ascospores, and by lacking atranorin.

Hypogymnia subfarinacea X.L. Wei & J.C. Wei, sp. nov. **Plate I:** C-E

Habitu cum Hypogymnia farinacea optime congruens, sed differt lobis pruinosis et acidum physodalicum continens.

Type: China. Sichuan Province, Nanping County, Jiuzhai Gou, alt. 2151 m, on trunk of *Tsuga* sp., 10 June 1983, X. Y. Wang & X. Xiao 10582 (holotype, HMAS-L).

Thallus foliose, loosely appressed, with subdichotomously branched and separated lobes of 2 mm wide; upper surface gray, dull, slightly rugose to smooth with some pieces of upper cortex in the old lobes disintegrating, lacking isidia and lobules but bearing granular soredia coalescent in more or less sacciform structures, with thin layer of pruina limited to the lobe tips; lower surface black, pale brown near the apices, wrinkled, glossy, with large, round perforations of 2 mm in diameter.

Upper cortex prosoplectenchymatous, pale yellow, 14.5 μm thick; algal layer 20.5-22.5 μm thick, consisting of green and subspherical cells of 3-4 μm in diameter; hyphae in medulla colorless, septate, 1-2 μm in diameter; lower cortex prosoplectenchymatous, pale yellow, 12-14.5 μm thick. Apothecia and pycnidia unknown.

Chemistry. Cortex K-, C-, P-; medulla K+ yellow, C-, P+ orange yellow → orange red, containing physodalic, physodic, 3-hydroxyphysodic (conphysodic), protocetraric acids, and atranorin.

Other Material Examined: **China, Yunnan:** Lijiang County, Mt. Yulong shan, alt. 2900 m, on the bark of *Quercus* sp., 8 August 1981, X. Y. Wang, X. Xiao & J. J. Su 4892 (HMAS-L); alt. 3100 m, on the ground, 8 August 1981, X. Y. Wang, X. Xiao & J. J. Su 6591 (HMAS-L).

Comments: The new species resembles *H. farinacea* Zopf at the first sight, but differs by more separated lobes, presence of pruina limited to the lobe tips, and in containing physodalic acid.

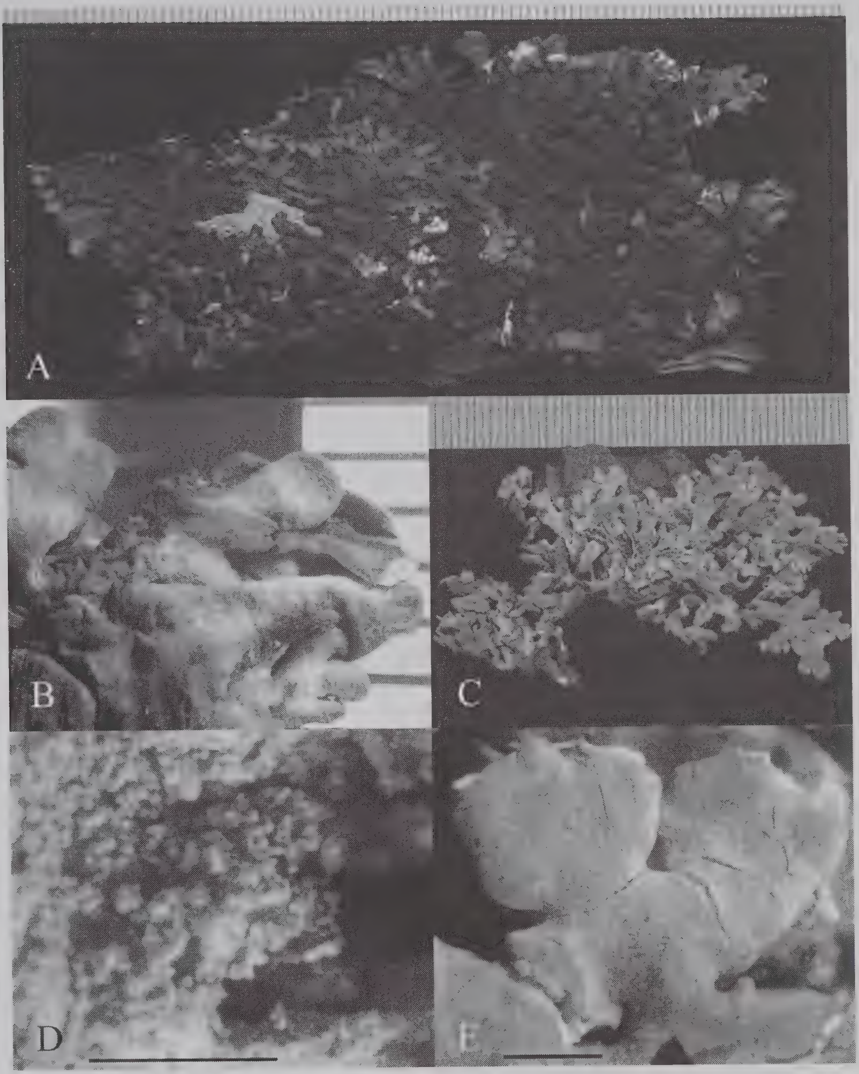


Plate I. A. *Hypogymnia pseudopruinosa*, Wang et al. *7606* (holotype in HMAS-L), showing general appearance of thallus. B. *H. pseudopruinosa*, Wang et al. *7606*, showing the pruina limited to the margin of lobes. C. *H. farinacea*, Wang et al. 10582 (holotype in HMAS-L), showing general appearance of thallus. D. *H. farinacea*, Wang et al. 10582, showing the pruina limited to the margin of lobes. E. *H. farinacea*, Wang et al. 10582, showing the soredia on the upper surface.

A-C: Scale in mm; D-E: Scale bar = 1 mm.

Acknowledgements

We are indebted to Prof. Teuvo Ahti and Dr. Harrie Sipman for giving valuable comments on the manuscript. Special thanks due to Ms. H. Deng for giving considerable assistance during the studies in HMAS-L. The authors are also grateful to Mr. Q. M. Zhou, and Drs. M. R. Huang and S. Y. Guo for kind help in taking the pictures.

Literature Cited

Chen JB. 1994. Two new species of *Hypogymnia* (Nyl.) Nyl. (*Hypogymniaceae*, Acomycotina). Acta Mycologica Sinica 13 (2): 107-110.

Culberson CF. 1972. Improved conditions and new data for the identification of lichen products by a standardized thin-layer chromatographic method. Journal of Chromatography 72: 113-125.

Culberson CF, Kristinsson H. 1970. A standardized method for the identification of lichen products. Journal of Chromatography 46: 85-93.

McCune B, Martin EP, Wang LS. 2003. Five new species of *Hypogymnia* with rimmed holes from the Chinese Himalayas. The Bryologist 106 (2): 226-234.

McCune B, Obermayer W. 2001. Typification of *Hypogymnia hypotrypa* and *H. sinica*. Mycotaxon 79: 23-27.

McCune B, Tchabanenko S. 2001. *Hypogymnia arcuata* and *H. sachalinensis*, two new lichens from east Asia. The Bryologist 104 (1): 146-150.

Wei JC. 1991. An Enumeration of Lichens in China. International Academic Publishers.

White FJ, James PW. 1985. A new guide to microchemical techniques for the identification of lichen substances. British Lichen Society Bulletin 57 (Suppl.): 1-41.

Zhao JD. 1964. A preliminary study on Chinese *Parmelia*. Acta Phytotaxonomica Sinica 9: 139-166.

MYCOTAXON

Volume 94, pp. 159–173 October–December 2005

Discovery and description of a teleomorph for *Leptographium koreanum*

H. Masuya[1], J.-J. Kim[2], M. J. Wingfield[3], Y. Yamaoka[4],
S. Kaneko[1], C. Breuil[2], & G.-H. Kim[5]

H_masu@hotmail.com
[1] Forest Pathology Laboratory, Forestry and Forest Products Research Institute
Tsukuba, Ibaraki 305-8687, Japan

[2] Department of Wood Science, University of British Columbia
Vancouver, B.C. V6T 1Z4, Canada

[3] Forestry and Agricultural Biotechnology Institute (FABI)
University of Pretoria, Pretoria 0002, South Africa

[4] Graduate School of Life and Environmental Science, University of Tsukuba
Tsukuba, Ibaraki 305-8572, Japan

[5] Division of Environmental Science & Ecological Engineering
Korea University, Seoul 136-701, Korea

Abstract—A *Leptographium* sp. unknown in Japan was isolated from Japanese red pine (*Pinus densiflora*) in this study. Pairing of different strains of the fungus gave rise to mature perithecia of an *Ophiostoma* sp. Characteristics of this teleomorph were similar to those of *Ophiostoma piceaperdum*, but the fungus had larger ascospores. The anamorph state had 2 to 3 primary branches, and was similar to *Leptographium truncatum*, *L. yunnanense*, *L. pini-densiflorae*, and particularly to *L. koreanum*. Comparisons of partial actin, β-tubulin, and rDNA sequences data showed that the Japanese fungus is *L. koreanum*. Pairing of strains from Japan with the ex-type culture of *L. koreanum* gave rise to mature perithecia confirming this identification. The teleomorph of *L. koreanum* is thus described here as *Ophiostoma koreanum*.

Key words—biological species, phylogenetic species, ophiostomatoid fungi, blue-stain fungi, DNA sequences

Introduction

Species of *Leptographium* Lagerb. et Melin are best recognized as anamorphs of the ascomycete genus *Ophiostoma* Syd. et P. Syd. They include economically important agents of sap-stain as well as tree pathogens (Gibbs 1993, Seifert 1993). *Leptographium wingfieldii* M. Morelet and *L. wageneri* (W. B. Kendr.) M. J. Wingf. have relatively high levels of virulence, and the three varieties of the latter species are well-recognized root pathogens in the western United States (Cobb 1998, Solheim et al. 1993, Harrington 1988). These and other *Leptographium* spp., produce mitospores in slimy masses at the apices of erect conidiophores that are specifically adapted to be carried by arthropods, particularly bark beetles (Coleoptera, Scolytinae, Jacobs and Wingfield 2001).

A recent monograph on *Leptographium* included 46 species (Jacobs & Wingfield 2001). The majority of these species occur in Europe and North America and it is well-recognized that this group of fungi has been poorly sampled in other parts of the world. This is particularly true for Asia where a large number of bark beetles are known to infest native conifers. This study represents part of an ongoing effort to catalogue the *Leptographium* spp. in Asia, and particularly in Japan.

Recent isolations from pine bark beetles and bark beetle-infested Japanese red pine (*Pinus densiflora* Siebold et Zucc.) and other pine species have yielded a *Leptographium* spp. that has previously not been collected in Japan. This fungus has 2-3 primary branches, relatively poorly developed rhizoids, and is morphologically similar to *L. truncatum* (M. J. Wingf. et Marasas) M. J. Wingf., *L. yunnanense* X. D. Zhou et al., *L. koreanum*, and *L. pini-densiflorae* Masuya et M. J. Wingf. (Jacobs & Wingfield 2001, Kim et al. 2005, Masuya et al. 2000, Zhou et al. 2000, Jacobs et al. 2005). However, unlike these fungi, isolates of the Japanese *Leptographium* sp. often produce protoperithecia in culture (Masuya et al. 1998). Masuya et al.(1998, 1999) have previously noted the fungus and reported it as an undescribed *Ophiostoma* sp. This fungus was isolated from 9 out of 13 investigated bark beetle species and appears to be widely distributed in Japan (Masuya et al. 2001). It was also found to be relatively virulent in inoculations on Japanese red pine where it produced longer lesions than various other blue-stain fungi (Masuya et al. 2003).

The unknown *Ophiostoma* sp. with a *Leptographium* asexual state from Japan is considered to be economically important for Japan thus an appropriate name for the fungus is required especially since its teleomorph is recognized. The aim of this study is to establish its identity.

Materials and methods

Fungal isolates

The fungal isolates used for morphological comparisons and mating experiments in this study are listed in Table 1. Five strains of the *Leptographium* sp. (MCC206, 214, 217, 364, 365) were used in the DNA sequence analyses. Comparisons of sequence data also included sequence data of other *Ophiostoma* and *Leptographium* species obtained from Genbank (Table 2).

Morphology

A 5 mm-diam. plug of each isolate used in the morphological comparisons was placed in Petri dishes containing 2% malt extract agar (MEA, 20 g Difco malt extract, 15 g agar and 1000mL distilled water) and incubated at 20C in dark. After one month, two autoclaved pine twigs were placed on the surface of the medium to stimulate the development of fruiting structures. After an additional month of incubation, plates were inspected for the presence of perithecia. Where these structures were found, they were mounted on glass slides in 1% lacto-fucsin for microscopic examination. In addition, perithecia were mounted after having been bleached with Sodium hypochlorite (1% available chlorine) for detailed observation of the cell arrangement of perithecial

Table 1. Isolates used for morphological comparisons and mating experiments in this study.

Species	Isolate No.*	Other No. *	Source	Collector	Origin
Leptographium sp.	MCC206	DAOM234395, JCM11853, MAFF410963	*Tomicus piniperda* on *Pinus densiflora*	H. Masuya	Japan
	MCC211	JCM11857, MAFF410964	*T. piniperda* on *P. densiflora*	H. Masuya	Japan
	MCC213	JCM11855, MAFF410965	*T. piniperda* on *P. densiflora*	H. Masuya	Japan
	MCC214	DAOM234396, JCM11854, MAFF410966	*T. piniperda* on *P. densiflora*	H. Masuya	Japan
	MCC215	JCM11859, MAFF410967	*T. piniperda* on *P. densiflora*	H. Masuya	Japan
	MCC217	JCM11860, MAFF410968	*T. piniperda* on *P. densiflora*	H. Masuya	Japan
	MCC364	JCM11858, MAFF410961	*Hylurgops interstitialis* in *P. parviflora* var. *pentaphylla*	H. Masuya	Japan
	MCC365	JCM11856, MAFF410962	*H. interstitialis* in *P. parviflora* var. *pentaphylla*	H. Masuya	Japan
L. lundbergii	CBS352.29	CMW217, PREM50548	*P. sylvestris*	T. Lagerberg/E. Melin	Sweden
L. truncatum	CMW21	PREM45896	Trunk of *P. radiata*	M. J. Wingfiled	New Zealand
	CMW28	ATCC58099	Root of *P. taeda*	M. J. Wingfiled	South Africa
	CMW30	PREM45699	Trunk of *P. strobus*	M. J. Wingfiled	New Zealand
L. koreanum	KUC2072	DAOM234393	*P. densiflora* infested with *T. piniperda*	J. –J. Kim & G. –H. Kim	Korea
	KUC2102	DAOM234392	*P. koraiensis* infested with *T. piniperda*	J. –J. Kim & G. –H. Kim	Korea
L. pini-densiflorae	MCC071	JCM10479, MAFF410861, CMW5157	*P. densiflora* infested with *T. piniperda*	H. Masuya	Japan
	MCC194	JCM10480, MAFF410865, CMW5158	*P. densiflora* infested with *T. piniperda*	H. Masuya	Japan
L. yunnanense	CMW5304		*T. piniperda* on *P. yunnanensis*	XD Zhou	China

* Culture collection source: ATCC, American Type Culture Collection, U.S.A; CBS, the culture collection of Centraalbureau voor Schimmelcultures, Utrecht, the Netherlands; CMW, culture collection of the Tree Pathology Co-operative Programme, Forestry and Agricultural Biotechnology Institute, University of Pretoria, South Africa; DAOM, National Mycological Herbarium, Canada; JCM, Japan Collection of Microorganisms, RIKEN BioResource Center, Japan; KUC, the Korea University Culture Collection, Korean University, Korea; MAFF, Genetic Resources Center, Culture Collection of National Institute of Agrobiological Resources, Japan; MCC, culture collection of the senior author.

Table 2. Isolates used for molecular comparison.

Species	Isolate No.[*1]	Origin	GenBank No.		
			ITS2 & LSU	Actin	β-tubulin
Leptographium sp.	MCC206	Japan	AB222065[*2]	AB222061[*2]	AB222063[*2]
	MCC214	Japan	AB222066[*2]	AB222062[*2]	AB222064[*2]
	MCC217	Japan			
	MCC364	Japan			
	MCC365	Japan			
L. koreanum	KUC2072	Korea	AY707196	AY707174	AY707183
	KUC2102	Korea	AY707197	AY707175	AY707184
L. lundbergii	CBS352.29	Sweden	AY707198	AY707176	AY707185
L. pini-densiflorae	MCC071	Japan	AY707199		AY707186
	MCC194	Japan	AY707200		AY707187
L. pyrinum	DLS879	USA	AY544604	AY544586	AY263185
L. terebrantis	C418	USA	AY544607	AY544589	AY263191
L. truncatum	CMW21	New Zealand	DQ062056		DQ061990
	CMW28	South Africa	DQ062052		DQ061986
	CMW30	New Zealand	DQ062054		DQ061988
L. wingfieldii	CMW2095	France	AY707204	AY707177	AY707190
L. yunnanense	CMW5304	China	AY707206	AY707179	AY707192
Ophiostoma aenigmaticum	CMW2199	Japan	AY553389		
	CMW2310	Japan	AY553390		
O. aureum	ATCC16936	Canada	AY544610	AY544592	AY263187
O. clavigerum	ATCC18086	Canada	AY544613	AY544595	AY263194
O. huntii	UAMH4997	Canada	AY544617	AY544599	AY349023
O. laricis	CMW1980	Japan	AF343691		
O. piceaperdum	C274	USA	AY707209	AY707182	AY707195
O. robustum	CMW668	USA	AY544619	AY544601	AY263185

[*1] Culture collection source: ATCC, American Type Culture Collection, U.S.A; C, Collection of T. C. Harrington, Iowa State University; CBS, the culture collection of Centraalbureau voor Schimmelcultures, Utrecht, the Netherlands; CMW, culture collection of the Tree Pathology Co-operative Programme, Forestry and Agricultural Biotechnology Institute, University of Pretoria, South Africa; DLS, culture collection of D. L. Six; KUC, the Korea University Culture Collection, Korean University, Korea; MCC, culture collection of the senior author; UAMH, University of Alberta Microfungus Collection and Herbarium, Devonian Botanic Garden, Edmonton.

[*2] Accession numbers of the sequences obtained in this study.

necks and outer layers of the peridium. Morphologically characteristic structures were measured and averages and ranges computed. Fifty measurements for each structure were made.

Mating experiments

Isolates of the unknown *Leptographium* sp. were paired in all possible combinations. In addition, four isolates originating from single ascospores derived from a single perithecium from a cross between isolates MCC206 and MCC214, were paired with each other in all possible combinations. We use MCC206 and MCC214 as tester isolates and try to pair each one with other species listed in Table 1. Negative control mating experiments also were done.

Plugs from 2-wk-old cultures on 2% MEA were excised with cork borer (5mm diam.) and placed on 2% MEA. Plates were incubated at 15 °C in the dark for 2 weeks. Two autoclaved pine twigs or sapwood blocks were then placed on the agar surface and the plates were incubated for an additional 2 weeks. Donor cultures were flooded with 600ml sterile and deionized water and conidial suspensions were prepared. This suspension was poured on the twigs in the each recipient culture. Plates were then incubated at 15 C and these were regularly inspected over a period of two months for the mature perithecia.

DNA sequence comparisons

Cultures for DNA sequence comparisons were incubated on 2% MEA plates for four weeks. DNA was extracted using the methods described by Kim et al. (2005). Oligonucleotide primers used for both amplification and DNA sequencing of the internal transcribed spacer (ITS) 2 and partial large subunit (LSU) regions of the ribosomal DNA operon, portions of the actin and β-tubulin genes were the same as those used by Kim et al. (2005). Methods for amplification and sequencing of each gene or gene region were also as described by Kim et al. (2005). Both strands of fragments were sequenced and sequences have been deposited in GenBank (Table 2, Fig. 1).

Obtained sequences were analyzed together with previously published sequences to provide a sufficiently broad taxon sampling (Table. 2). Overall, the ITS2 and LSUrDNA—D1 sequence data set included 23 sequences including those derived in this study. The actin and β-tubulin gene sequence data set was comprised of 16 and 22 sequences including those obtained in this study.

Sequences were aligned using Clustal X version 1.81 (Thompson et al. 1997). Alignments were manually adjusted using the program BioEdit version 5.0.9 (Hall 1999). The aligned data set was analyzed using the program PAUP*4.0 beta10 (Swofford 2002). A parsimony analysis was carried out using the heuristic search with simple stepwise addition, MAXTREE option set to 1000, and tree-bisection reconnection (TBR) option of the program. Gaps were treated as missing data and all characters were equally weighted. Bootstrap and jackknife values (each 1000 replicates) were also calculated.

Results

Morphology

The unknown *Leptographium* sp. from Japan was characterized by having mainly two primary branches and conidia with truncated bases. These characteristics are commonly found in various species of *Leptographium*. In particular, *L. truncatum*, *L. pini-densiflorae*, *L. koreanum*, and the *Leptographium* anamorphs of *Ophiostoma* spp. including *L. laricis* Van der Westh. et al., *L. aenigmaticum* K. Jacobs et al., *L. huntii* M. J. Wingf. and *L. piceaperdum* K. Jacobs & M. J. Wingf. have similar characters to those of the unknown *Leptographium* sp. from Japan. However, the stipe lengths of the unknown *Leptographium* sp. were longer than those of *L. piceaperdum*, *L. laricis* and *L. aenigmaticum*. Hyphal characteristics of the Japanese fungus were also different from those of *L. huntii*, which are typically serpentine, but could not be distinguished from all other species considered in this study. Primary branches of the Japanese *Leptographium* sp. were smaller than those of *L. truncatum*. Conidia of the *Leptographium* sp. were the

Table 3. Teleomorphic characters of the *Leptographium* species and morphologically similar species.

Character	*Leptographium* sp.	*O. piceaperdum*[a]	*O. laricis*[b]	*O. aenigmaticum*[c]	*O. huntii*[d]
Perithecial diam.	240–310	(170–) 199–312 (–370)	210–310	142–254	280–448
Perithecial neck length	520–1000	(280–) 503–603(–850)	400–1320	115–310	140–720
Perithecial neck width at the base	52–75	(30–) 32–60	50–70	35–100	40–70
Perithecial neck width near the tip	25–33	20–30	20–50	20–45	21–42
Ascospore shape	Hat-shaped to cucullate	Hat-shaped	oblong to ellipsoid	Hat-shaped with elongated brims	Hat-shaped
Ascospore size	5.5–10.5 × 4.5–7.5	(3–) 4–5 × 2–3	6–11 × 2–4	4–5 × 1.8–3.5	3–4 × 1.5–2
Shape of perithecial outer wall composed cell	polygonal to irregularly shaped	–	–	–	elongate to oval
Size of perithecial outer wall composed cell	10–22 × 8–21	–	–	–	ca. 19.6 × 9.7

[a] Jacobs & Wingfield (2001), [b] Van der Westhuizen et al. (1995), [c] Jacobs et al. (1998), [d] Robinson-Jeffrey & Grinchenko (1964)

Table 4. Result of mating experiment of the Leptographium sp.

		recipient																
	donor	MCC206	MCC211	MCC213	MCC214	MCC215	MCC217	MCC364	MCC365	KUC2102	KUC2072	CBS352.29	CMW21	CMW28	CMW30	MCC071	MCC194	CMW5304
Leptographium sp.	MCC206	-	-	-	++	-	++	-	-	+	+	-	-	-	-	-	-	-
	MCC211	-	-	+	-	-	-	-	-	NT	NT	-	-	-	-	-	-	NT
	MCC213	-	+	-	++	-	-	+	-	NT	NT	-	-	-	-	-	-	NT
	MCC214	++	-	+	-	-	-	-	++	-	-	-	-	-	-	-	-	-
	MCC215	-	-	-	+	-	-	-	-	NT	NT	-	-	-	-	-	-	NT
	MCC217	-	-	+	-	-	-	-	+	NT	NT	-	-	-	-	-	-	NT
	MCC364	-	-	++	-	-	-	-	++	-	-	-	-	-	-	-	-	-
	MCC365	-	-	-	++	-	+	-	-	-	-	-	-	-	-	-	-	-
	KUC2102	+	NT	NT	-	NT	NT	-	-	NT	NT	NT	NT	NT	NT	NT	NT	NT
	KUC2072	+	NT	NT	-	NT	NT	-	-	NT	NT	NT	NT	NT	NT	NT	NT	NT
L. lundbergii	CBS352.29	-	-	-	-	-	-	-	-	NT	NT	-	-	-	-	-	-	NT
L. truncatum	CMW21	-	-	-	-	-	-	-	-	NT	NT	-	-	-	-	-	-	NT
	CMW28	-	-	-	-	-	-	-	-	NT	NT	-	-	-	-	-	-	NT
	CMW30	-	-	-	-	-	-	-	-	NT	NT	-	-	-	-	-	-	NT
L. pini-densiflorae	MCC071	-	-	-	-	-	-	-	-	NT	NT	-	-	-	-	-	-	NT
	MCC194	-	-	-	-	-	-	-	-	NT	NT	-	-	-	-	-	-	NT
L. yunnanense	CMW5304	-	NT	NT	-	NT	NT	-	-	NT	NT	NT	NT	NT	NT	NT	NT	NT

*– no perithecia produced, +: perithecia (n <50) produced, ++: perithecia (n >50) produced, NT: not tested.

same as those of *L. koreanum*, which are oblong to ovoid and relatively straight and thus, different to those of other species.

The morphology of the teleomorph structures produced as a result of the pairing of various strains was similar to those of *Ophiostoma piceaperdum* (Rumbold) Arx, *O. aenigmaticum* K. Jacobs et al., and *O. huntii* (Rob.-Jeffr.) de Hoog & R. J. Scheff. where ascospores were hat-shaped and perithecial necks had no ostiolar hyphae. Perithecia sizes and neck lengths overlapped between species and could not be clearly separated. However, the ascospores of the unidentified *Ophiostoma* sp. were larger than those of the other species with which it might be confused (Table 3).

Mating experiments

Two isolates (MCC206 and MCC214) of the unidentified *Leptographium* sp. from Japan produced protoperithecia in culture, but none of them produced mature perithecia unless they were paired with other isolates. Pairing of isolates with themselves did not result in perithecia. The results of the pairing of isolates in all possible combinations showed that some isolates were able to produce perithecia (Table 4). In addition, an isolate of the Japanese *Leptographium* sp. produced perithecia, when it was crossed with the ex-type strain of *L. koreanum* (KUC2102), and not when it was paired with other fungal species such as *L. truncatum* and *L. pini-densiflorae*. The result of crosses between single ascospore isolates showed clearly that the mating behavior of this fungus was heterothallic with two mating types.

DNA sequence comparisons

Phylogenetic analyses of sequences for the ITS2 and partial LSU of rDNA gene regions showed that isolates of the unknown *Leptographium* sp. from Japan is clearly distinct from *O. piceaperdum, O. laricis, O. aenigmaticum* and *L. pini-densiflorae* but that it reside a monophyletic group with isolates of *L. koreanum*, *L. truncatum*, *L. yunnanense* and other *Ophiostoma* spp. and *Leptographium* spp. (Fig. 1a). This result was supported by strong bootstrap/Jackknife values (83/78). From a total of 610 characters, 572 characters were constant, 13 variable characters were parsimony uninformative and 25 were informative. The heuristic search gave rise to 390 most parsimonious trees, of which one was chosen for presentation (Fig. 1a). The tree had a length of 44 steps with a Consistency Index (CI) of 0.9091, a Homoplasy Index (HI) of 0.0909, and a Retention Index (RI) of 0.9565.

Phylogenetic analysis of the β-tubulin gene sequences resulted in the most parsimonious tree shown in Fig. 1b. This analysis also showed that the unknown *Leptographium* grouped with high bootstrap/Jackknife support (92/82) in the clade containing *L. koreanum*, and not with *L. truncatum*, *L. yunnanense*, *L. lundbergii*, *O. piceaperdum*, and *O. aenigmaticum*. From a total of 360 characters, 235 characters were constant, 19 variable characters were parsimony-uninformative and 106 were informative. The heuristic search found 87 most parsimonious trees of which one was chosen for presentation (Fig. 1b). The tree had a length of 221, a Consistency Index (CI) of 0.8190, a Homoplasy Index (HI) of 0.1810, and a Retention Index (RI) of 0.8987.

The phylogenetic analysis of the actin gene sequences showed that the unknown *Leptographium* sp. from Japan resided in a monophyletic group with *L. koreanum* (Fig. 1c) with high bootstrap/Jackknife support (99/98). *Leptographium yunnanense*

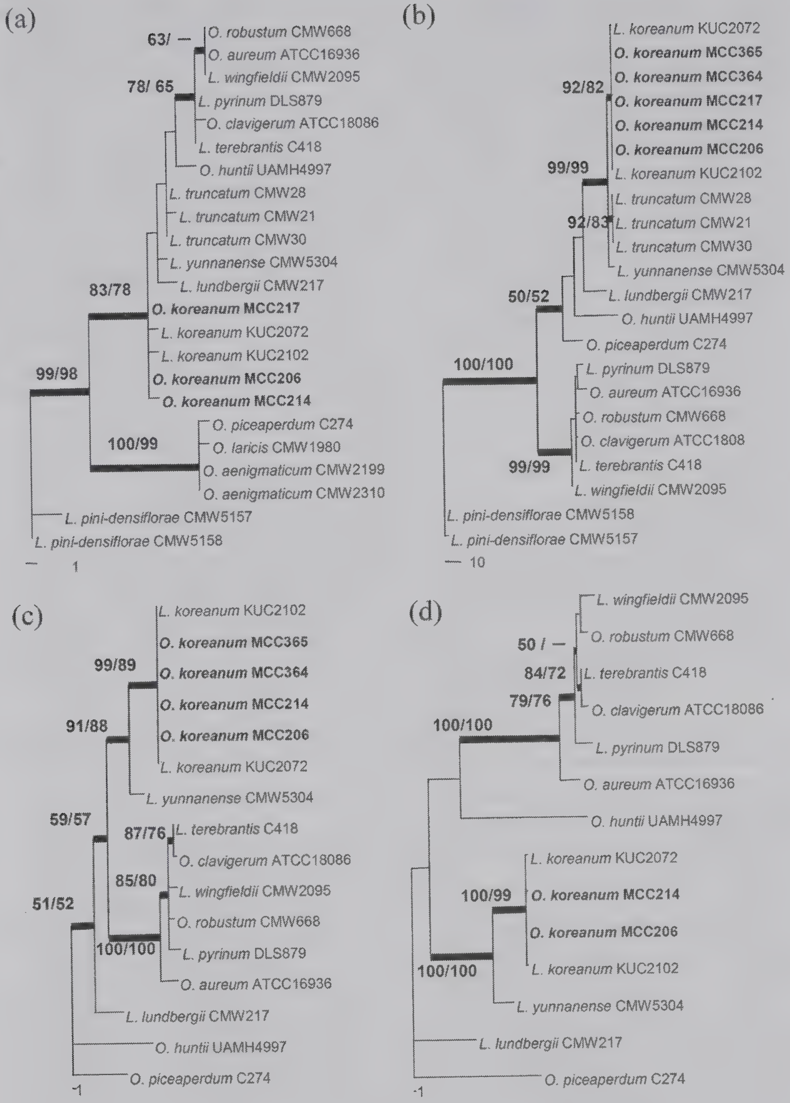

(a)
63/ —
O. robustum CMW668
O. aureum ATCC16936
L. wingfieldii CMW2095
78/ 65
L. pyrinum DLS879
O. clavigerum ATCC18086
L. terebrantis C418
O. huntii UAMH4997
L. truncatum CMW28
L. truncatum CMW21
L. truncatum CMW30
L. yunnanense CMW5304
L. lundbergii CMW217
83/78
O. koreanum MCC217
L. koreanum KUC2072
L. koreanum KUC2102
99/98
O. koreanum MCC206
O. koreanum MCC214
O. piceaperdum C274
100/99
O. laricis CMW1980
O. aenigmaticum CMW2199
O. aenigmaticum CMW2310
L. pini-densiflorae CMW5157
L. pini-densiflorae CMW5158
1
(b)
L. koreanum KUC2072
O. koreanum MCC365
O. koreanum MCC364
92/82
O. koreanum MCC217
O. koreanum MCC214
O. koreanum MCC206
99/99
L. koreanum KUC2102
L. truncatum CMW28
92/83
L. truncatum CMW21
L. truncatum CMW30
L. yunnanense CMW5304
50/52
L. lundbergii CMW217
O. huntii UAMH4997
O. piceaperdum C274
100/100
L. pyrinum DLS879
O. aureum ATCC16936
O. robustum CMW668
O. clavigerum ATCC1808
99/99
L. terebrantis C418
L. wingfieldii CMW2095
L. pini-densiflorae CMW5158
L. pini-densiflorae CMW5157
10
(c)
L. koreanum KUC2102
O. koreanum MCC365
99/89
O. koreanum MCC364
O. koreanum MCC214
91/88
O. koreanum MCC206
L. koreanum KUC2072
L. yunnanense CMW5304
59/57
87/76
L. terebrantis C418
O. clavigerum ATCC18086
85/80
L. wingfieldii CMW2095
51/52
O. robustum CMW668
100/100
L. pyrinum DLS879
O. aureum ATCC16936
L. lundbergii CMW217
O. huntii UAMH4997
O. piceaperdum C274
1
(d)
L. wingfieldii CMW2095
O. robustum CMW668
50 / —
84/72
L. terebrantis C418
79/76
O. clavigerum ATCC18086
100/100
L. pyrinum DLS879
O. aureum ATCC16936
O. huntii UAMH4997
L. koreanum KUC2072
100/99
O. koreanum MCC214
O. koreanum MCC206
100/100
L. koreanum KUC2102
L. yunnanense CMW5304
L. lundbergii CMW217
O. piceaperdum C274
1

Fig. 1. The most parsimonious trees for each of the three nuclear gene datasets and the combined datasets: (a) rDNA, (b) β-tubulin, (c) actin, and (d) combined. The tree is unrooted. Bootstrap and Jackknife values > 50% with 1000 replications are indicated at the left of the branches (bootstrap value/Jackknife value).

appeared in a sister clade to *L. koreanum*. From a total of 763 characters, 668 characters were constant, 51 variable characters were parsimony-uninformative and 44 were informative. The heuristic search gave rise to two most parsimonious trees of which one (Fig. 1c) is presented. The tree length was 132 with a Consistency Index (CI) of 0.8788, a Homoplasy Index (HI) of 0.1212, and a Retention Index (RI) of 0.9171.

A combined sequence data set was also produced including 14 sequences representing the ITS2 and LSUrDNA—D1 domain, and parts of the actin, and β-tubulin genes. As was true for the analyses of the sequence data for the individual genes or gene regions, the phylogenetic analyses (Fig. 1d) showed that the unknown *Leptographium* sp. from Japan resided in a monophyletic group with *L. koreanum*, with a high bootstrap/Jackknife values (100/99). In this tree (Fig. 1d), *L. yunnanense* resided in a sister group to *L. koreanum*. From a total of 1728 characters, 1510 characters were constant, 110 variable characters were parsimony-uninformative and 108 were informative. The heuristic search found two most parsimonious trees of which one was chosen for presentation. The tree length was 305 with a Consistency Index (CI) of 0.8659, a Homoplasy Index (HI) of 0.1344, and a Retention Index (RI) of 0.9014.

Taxonomy

Results of the DNA sequence and morphological comparisons show clearly that the *Leptographium* anamorph of unknown *Ophiostoma* species from Japan is conspecific with *L. koreanum*. Furthermore, crosses between isolates of this fungus and those of *L. koreanum* have given rise to its *Ophiostoma* teleomorph. Teleomorph characteristics are also distinct from other *Ophiostoma* spp. with *Leptographium* anamorphs. Thus, on the basis of the mating behavior, morphological characteristics and DNA sequence comparisons, we described the teleomorph of *L. koreanum* as follows:

Ophiostoma koreanum Masuya, J.-J. Kim & M. J. Wingf. **sp. nov.** **Figs. 2-7.**

Anamorph: *Leptographium koreanum* J.-J. Kim & G.-H. Kim, Mycol. Res. 109(3): p. 275, 2005.

Perithecia basi nigra, globosa vel subglobosa, 240—310 μm diam, Collum cylindraceum, curvatum vel rectum, 560—1000 μm longum, ad basim 52—75 μm latum. ad apicem 25—33 μm latum, apice obtusum vel truncatum, hyphis ostioli non praeditum. Asci evanescenti. Ascosporae hyalinae, aseptatae, aspectu laterali cuculatae, aspectu frontali triangulatae, vagina hyalina circumdantes, 5.5—10.5 × 4.5—7.5μm.

Etymology: Derived from the name of anamorph, *Leptographium koreanum*.

Perithecia superficial or partly embedded in the substratum and medium. Basal part black, globose to subglobose, 240—310 (mean 290) μm diam. without hyphal ornamentation, outer layer of the peridium composed of thick-walled, more or less isodiametric, polygonal or irregularly shaped cells, 10—22 × 8—21 (mean 18 × 12) μm. Necks dark brown to black, broad at the base, becoming cylindrical or slightly tapered at the tip, straght or curved, 560—1000 (mean 680) μm long, 52—75 (mean 68) μm wide at base, 25—33 (mean 30) μm wide near the tip, composed of dark, thick-walled, squamous cells, 4—10 × 2—4 μm, terminating in an obtuse to truncate apex. Ostiolar hyphae absent. Asci evanescent, clavate when young, subglobose when mature, up to 25 × 20 μm. **Ascospores**, hyaline, one-celled, hat-shaped or cucullate in side view, triangular

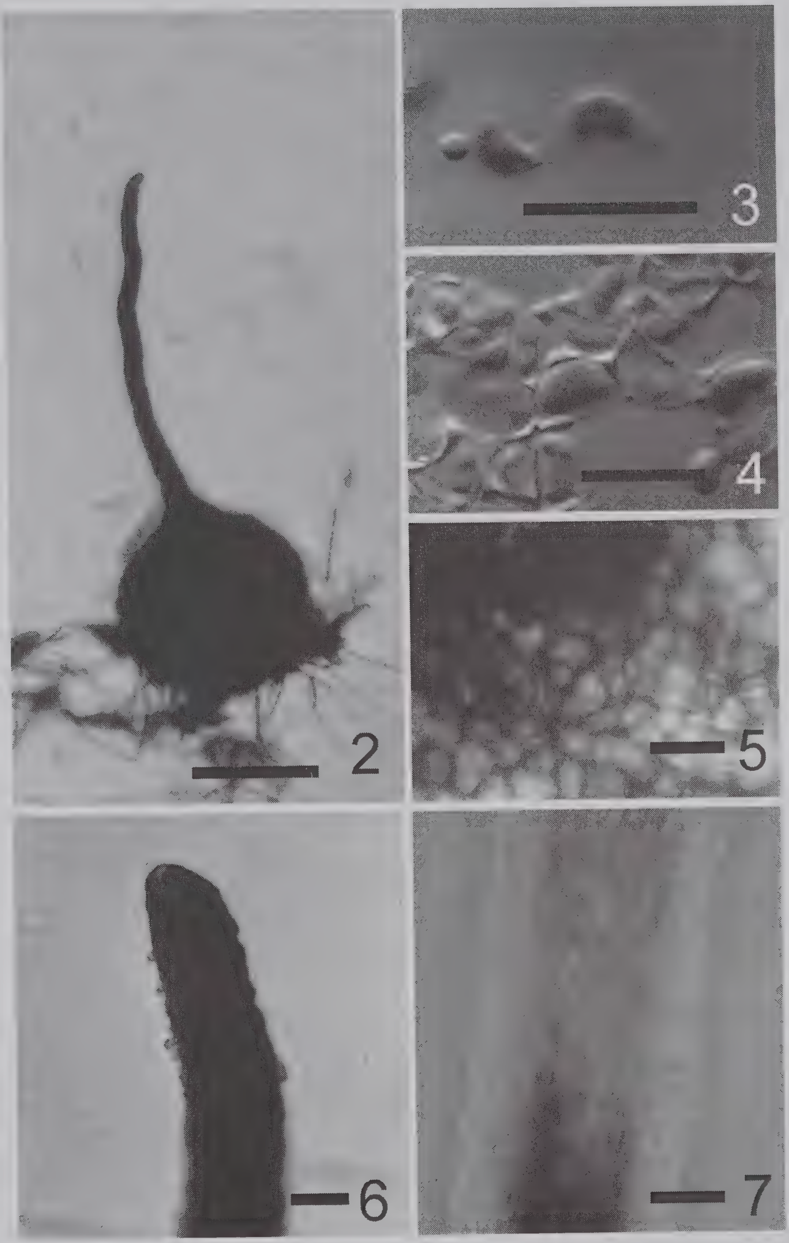


Fig. 2—7. *Ophiostoma koreanum*. Fig. 2. Perithecium. Fig. 3. Ascospore. Fig. 4. Ascospores. Fig. 5. Outer layer of the peridium composed of thick-walled, more or less isodiametric, polygonal or irregularly shaped cells. Fig. 6. Tip of perithecial neck. Fig. 7. Perithecial neck, composed of dark, thick-walled, squamous cells. Bars: Fig. 2 = 100μm, Figs. 3—5, 7 = 10μm, Fig. 6 = 5μm.

in front view, 5.5—10.5 × 4.5—7.5 (mean 7.5 × 5.5) μm, surrounded by a distinct hyaline wall, sometimes accumulating in a white mass at the tips of neck.

Morphology of anamorph is as described for *L. koreanum* (Kim et al. 2005).

Hosts: *Pinus densiflora*, *P. koraiensis* Siebold & Zucc. and other *Pinus* spp.

Isolated from following insects: *Tomicus piniperda* L., *Hylurgops interstitialis* (Chapuis), *Hylastes paralleus* Chapuis, *Hylastes plumbeus* Blandford and other bark beetle species.

Known distribution: Korea, Japan.

CULTURES AND SPECIMENS EXAMINED—HOLOTYPE: DAOM234414, dried culture derived from the pairing of the cultures KUC2102 (MAT-1) (DAOM234392) (KOREA. YEOJU: Sawmill, Central Forest Products Processing & Marketing Center, *P. koraiensis* log, 10 Aug 2000, *J.-J. Kim & G.-H. Kim*) and MCC206 (MAT-2) (DAOM234395, JCM11853, MAFF410963) (JAPAN. IBARAKI: Tsukuba, *T. piniperda* adult, 4 Apr 1995, *H. Masuya*) on *P. contorta* Dougl. sapwood block. **PARATYPES**: FPH (= TFM) 7605, dried culture from pairing between isolates MCC206 (MAT-2) (DAOM234395, MAFF410963) and MCC214 (MAT-1) (DAOM 234396, JCM11854, MAFF410966) (JAPAN. YAMANASHI: Masuho, *T. piniperda* adult, 15 May 1996, *H. Masuya*) on *P. densiflora* twigs, FPH (TFM) 7606, dried culture from pairing between isolates MCC213 (MAT-2) (JCM11855, MAFF410965) (JAPAN. FUKUSHIMA: Amasakae, *T. piniperda* adult, 24 Jun 1996, *H. Masuya*) and MCC214 (MAT-1) on *P. densiflora* twigs, FPH (TFM) 7607, dried culture from pairing between isolates MCC365 (MAT-2) (JCM11856, MAFF410962) (JAPAN. IWATE: Matsukawa, *H. interstitialis* adult, 1 Jun 2000, *H. Masuya*) and MCC214 (MAT-1) on *P. densiflora* twigs. **Additional cultures examined.** KOREA. YEOJU: Sawmill, Central Forest Products Processing & Marketing Center, *P. koraiensis* log, 10 Aug 2000, *J.-J. Kim & G.-H. Kim* (KUC 2072, MAT-1, DAOM 234393, CMW14199). Bongwha: Sawmill, National Forestry Cooperatives Federation, *P. densiflora* log, 9 Aug 2000, *J.-J. Kim & G.-H. Kim* (KUC 2078, MAT-1, CMW 14201, PREM 58261). JAPAN. IWATE: Ichinoseki, *T. piniperda* adult, 19 Jun 1996, *H. Masuya* (MCC211, MAT-1, JCM11857, MAFF410964), HOKKAIDO: Yamabe, bark of *P. sylverstris* dead tree, 7 Nov 1996, *H. Masuya* (MCC215, MAT-2, JCM11859, MAFF410967), IBARAKI: Tsukuba, *T. piniperda* adult, 18 May 1995, *H. Masuya* (MCC217, MAT-1, JCM11860, MAFF410968), IWATE: Matsukawa, *H. interstitialis* adult, 1 Jun 2000, *H. Masuya* (MCC364, MAT-1, JCM11858, MAFF410961).

Discussion

Results of this study have shown that the unidentified *Leptographium* sp. commonly isolated from pine bark beetles and bark beetle-infested *Pinus* spp. in Japan is morphologically and phylogenetically identical to *L. koreanum*. *Leptographium koreanum* was originally isolated from Korean (*P. koraiensis*) and Japanese red (*P. densiflora*) pine in Korea and was described as a new species by Kim et al. (2005). This fungus was frequently isolated from *T. piniperda* and it appears to be an important causal agent of blue-stain of conifer timber in Korea.

In this study, we have shown that *L. koreanum* in Japan is able to form a teleomorph when sexually compatible isolates are crossed. We have thus described the teleomorph of the fungus as *O. koreanum*. This fungus has been known in Japan for more than a decade where is was reported as an undescribed *Ophiostoma* sp. by Masuya et al (1998, 1999), frequently associated with the pine shoot beetle, *T. piniperda*. The Japanese *Leptographium* strains could be also paired with the ex-type strain of *L. koreanum* confirming that these two are biological heterothallic species. The distribution and ecology of *L. koreanum* and *O. koreanum* thus appear to be similar in Korea and Japan.

The teleomorph of *L. koreanum* described in this study is characterized by hat-shaped ascospores and long necks without ostiolar hyphae. In this regard it is similar to that of *O. piceaperdum* sensu Jacobs et al. (2000) and related species. Taxonomic status of *O. piceaperdum*, however, remains questionable. Morphological comparisons of the dried type specimens led Jacobs et al. (2000) to treat *O. europhioides* (E. F. Wright & Cain) H. Solheim and *O. pseudoeurophioides* (Olchow. & J. Reid) Georg Hausner et al. as synonyms of *O. piceaperdum*. However, recent DNA based comparisons by Hausner et al. (2000) showed that *O. europhioides*, *O. pseudoeurophioides* and *O. piceaperdum* reside in different clades. This suggests that the species concept of *O. piceaperdum* sensu Jacobs et al (2000) deserves reconsideration. Indeed, Olchowecki & Reid (1974) showed that the *Leptographium* anamorph of *O. pseudoeurophioides* has curved conidia, unlike *O. piceaperdum* illustrated by Jacobs et al. (2000). Because the ex-type cultures of *O. pseudoeurophioides* and *O. piceaperdum* are no longer available, it is not possible to consider their morphological characteristics or to make DNA based phylogenetic comparisons. These studies are required but must await further collections.

Leptographium koreanum does not have curved conidia and its LSU rDNA sequences do not correspond with those of *O. pseudoeurophioides* (GenBank accession No. AF155678). These species are, therefore, clearly different. In addition, our DNA sequence comparisons have shown that *O. koreanum* is not related to well-defined culture of *O. piceaperdum*. We are thus confident that the new species does not represent *O. piceaperdum*.

Ophiostoma aenigmaticum was described by Jacobs et al. (1998) from Japan. This species had previously been treated as *O. europhioides* by Yamaoka et al. (1997) but was differentiated from *O. europhioides* based on the characteristic of elongated brims of the ascospores (Jacobs et al. 1998). Because *O. koreanum* does not have ascospores with elongated brims and because *O. aenigmaticum* is homothallic, as opposed to the heterothallic *O. koreanum*, these species can easily be distinguished from each other. In addition, they also differ in their hosts and insect vectors. DNA based comparisons in this study have also shown that *O. aenigmaticum* is more closely related to *O. piceaperdum* than to *L. koreanum*.

The heterothallic mating behavior appears to be relatively uncommon in *Ophiostoma* with *Leptographium* anamorphs. While heterothallism has been noted for many ascomycetes including those from the genus *Ophiostoma* (Brasier 1993), *O. huntii* is the only *Ophiostoma* with a *Leptographium* anamorph that has previously been shown to display this mating behaviour (Jacobs et al. 1998). This is in contrast to species such as *O. piceaperdum* sensu Jacobs et al. (2000), *O. europhioides*, and *O. aenigmaticum* that have been reported to be homothallic. The fact that *O. koreanum* is heterothallic clearly distinguishes it from related *Ophiostoma* species with *Leptographium* anamorphs.

A relatively small number of *Leptographium* spp. have known teleomorphs and the majority of these appear to have hat-shaped ascospores (Jacobs & Wingfield 2001). In some cases such as that of *Leptographium wageneri* var. *ponderosae*, perithecia thought to represent an *Ophiostoma* state have been found in galleries of beetle vectors but these have never been found in culture. This makes it difficult to confirm the anamorph/teleomorph connection. It is not uncommon to discover teleomorph states in fungi thought to exist only in the asexual form (Kuhls et al. 1996, Hodge et al. 1996, Chaverri et al. 2001). The discovery of the teleomorph of *L. koreanum* arising from crosses between different isolates suggests that some other *Leptographium* species might also be heterothallic and might have the ability to produce perithecia in crosses of sexually compatible isolates under suitable environmental conditions. This is clearly an area of research worth pursuing.

Acknowledgements

We thank Drs. T. Kubono, Y. Ichihara and M. Nakamura, Tohoku Research Center of Forestry & Forest Products Research Institute, Japan for technical help; Dr. K. Jacobs, University of Stellenbosch, South Africa for providing molecular data of several *Leptographium* species; Drs. A. Uzunovic, Canada's Wood Products Research Institute, Forintek Canda Corp., Canada and T. Kiristis, University of Natural Resources and Applied Life Sciences, Austria, for kind review of this manuscript.

Literature cited

Brasier CM. 1993. The genetic system as a fungal taxonomic tool: Gene flow, molecular variation and sibling species in the '*Ophiostoma piceae-Ophiostoma ulmi*' complex and its taxonomic and ecological significance. *In Ceratocystis* and *Ophiostoma*. Taxonomy, Ecology and Pathogenicity (eds. M. J. Wingfield, K. A. Seifert, and J. F. Webber), pp. 77-92. The American Phytopathological Society, St. Paul, Minnesota.

Chaverri P, Samuels GJ, Stewart EL. 2001. *Hypocrea virens* sp. nov., the teleomorph of *Trichoderma virens*. Mycologia 93:1113-1124.

Cobb FW. 1988. *Leptographium wageneri*, cause of black stain root disease, a review of its discovery, occurrence and biology with emphasis on pinyon and ponderosa pine. *In Leptographium* root disease in conifers (eds. Harrington TC, Cobb FW), pp. 41-62. The American Phytopathological Society, St Paul, Minnesota.

Gibbs JN. 1993. The biology of ophiostomatoid fungi causing sapstain in trees and freshly cut logs. *In Ceratocystis* and *Ophiostoma*. Taxonomy, Ecology and Pathogenicity (eds. M. J. Wingfield, K. A. Seifert, and J. F. Webber), pp. 141-152. The American Phytopathological Society, St. Paul, Minnesota.

Hall TA. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucl Acids Symp Ser 41:95-98.

Harrington TC. 1988. *Leptographium* species, their distributions, hosts and insect vectors. *In Leptographium* root disease in conifers (eds. Harrington TC, Cobb FW), pp. 1-39. The American Phytopathological Society, St Paul, Minnesota.

Hausner G, Reid J, Klassen R. 2000. On the phylogeny of members of *Ceratocystis s. s.* and *Ophiostoma* that possess different anamorphic states, with emphasis on the anamorph genus *Leptographium*, based on partial ribosomal DNA sequences. Can J Bot 78: 903-916.

Hodge KT, Krasnoff SB, Humber RA. 1996. *Tolypocladium inflatum* is the anamorph of *Cordyceps subsessilis*. Mycologia 88: 715-719.

Jacobs K, Wingfield MJ, Wingfield BD, Yamaoka Y. 1998. Comparison of *Ophiostoma huntii* and *O. europhioides* and description of *O. aenigmaticum* sp. nov. Mycol Res 102: 289-294.

Jacobs K, Wingfield MJ, Crous PW. 2000. *Ophiostoma europhioides* and *Ceratocystis pseudoeurophioides*, synonyms of *O. piceaperdum*. Mycol Res 104: 238-243.

Jacobs K, Wingfield MJ, Wingfield BD. 2001. Phylogenetic relationships in *Leptographium* based on morphological and molecular characters. Can J Bot 79: 719-732.

Jacobs K, Wingfield MJ. 2001. *Leptographium* species, tree pathogens, insect associates, and agents of blue-stain. The American Phytopathological Society, St. Paul, Minnesota, USA: APS press. 207p.

Jacobs K, Solheim H, Wingfield BD, Wingfield MJ. 2005. Taxonomic re-evaluation of Leptographium lundbergii based on DNA sequence comparisons and morphology. Myco Res 109: 1149-1161.

Kim J-J, Lim YW, Breuil C, Wingfield MJ, Zhou XD, Kim GH, 2005. A new *Leptographium* species associated with *Tomicus piniperda* infesting pine logs in Korea. Mycol Res 109: 275-284.

Kuhls K, Lieckfeldt E, Samuels GJ, Kovacs W, Meyer W, Petrini O, Gams W, Borner T, Kubicek CP. 1996. Molecular evidence that the asexual industrial fungus *Trichoderma reesei* is a clonal derivative of the ascomycete *Hypocrea jecorina*. Proc Natl Acad Sci USA 93:7755-7760

Masuya H, Kaneko S, Yamaoka Y. 1998. Blue stain fungi associated with *Tomicus piniperda* (Coleoptera: Scolytidae) on Japanese red pine. J For Res 3: 213-219.

Masuya H, Kaneko S, Yamaoka Y, Ohsawa M. 1999. Comparisons of ophiostomatoid fungi associated with *Tomicus piniperda* and *T. minor* in Japanese red pine. J For Res 4: 131-135.

Masuya H, Wingfield MJ, Kaneko S, Yamaoka Y. 2000. *Leptographium pini-densiflorae* sp. nov. from Japanese red pine. Mycoscience 41: 425-430.

Masuya H, Yamaoka Y, Kaneko S. 2001. Ophiostomatoid fungi isolated from Japanese red pine and their relationship with bark beetles. Proceeding of 3rd Regional Workshop of IUFRO working party 7.03.08, Forest Protection in Northeast Asia. Chiayi, Taiwan. p.193-198.

Masuya H, Kaneko S, Yamaoka Y. 2003. Comparative virulence among blue-stain fungi isolated from Japanese red pine. J For Res 8: 83-88.

Olchowecki A. Reid J. 1974. Taxonomy of the genus *Ceratocystis* in Manitoba. Can J Bot 52: 1675-1711.

Robinson-Jeffrey RC, Grinchenko AHH. 1964. A new fungus in the genus *Ceratocystis* occurring on blue-stained lodgepole pine attacked by bark beetles. Can J Bot 42:527-532.

Seifert KA. 1993. Sapstain of commercial lumber by species of *Ophiostoma* and *Ceratocystis*. In *Ceratocystis* and *Ophiostoma*. Taxonomy, Ecology and Pathogenicity (eds. MJ Wingfield, KA Seifert, JF Webber), pp. 141-151. The American Phytopathological Society, St. Paul, Minnesota.

Solheim H, Långström B, Hellqvist C. 1993. Pathogenicity of the blue-stain fungi *Leptographium wingfieldii* and *Ophiostoma minus* to Scots pine: effect of tree pruning and inoculum density. Can J For Res 23: 1438-1443.

Swofford DL. 2002. PAUP* 4.0 Phylogenetic analysis using parsimony, version 4.0b. Sunderland, Massachusetts: Sinauer Associates, Inc.

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG. 1997. The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nuc Acid Res, 24: 4876-4882.

Van der Westhuizen K, Wingfield MJ, Yamaoka Y, Kemp GHJ, Crous PW. 1995. A new species of *Ophiostoma* with *Leptographium* anamorph from larch in Japan. Mycol Res 99: 1334-1338.

Yamaoka Y, Wingfield MJ, Takahashi I, Solheim H. 1997. Ophiostomatoid fungi associated with the spruce bark beetle *Ips typographus* f. *japonicus* in Japan. Mycol Res 101: 1215-1227.

Zhou XD, Jacobs K, Morelet M, Ye H, Lieutier F. Wingfield MJ. 2000. A new *Leptographium* species associated with *Tomicus piniperda* in southwestern China. Mycoscience 41: 573-578.

MYCOTAXON

Volume 94, pp. 175–179 October–December 2005

Two parasitic fungi on a new host, *Syringa* (*Oleaceae*)

OVIDIU CONSTANTINESCU[1], VADIM A. MEL'NIK[2] & GERARD J.M. VERKLEY[3]

[1]*ovidiu.constantinescu@evolmuseum.uu.se*
Botany Section, Museum of Evolution, Uppsala University
Norbyvägen 16, SE-752 36 Uppsala, Sweden

[2]*Komarov Botanical Institute, Laboratory of Systematics and Geography of Fungi*
Prof. Popov Street 2, RU-197376 St. Petersburg, Russia

[3]*Fungal Biodiversity Centre, Centraalbureau voor Schimmelcultures*
Uppsalalaan 8, NL-3584 CT Utrecht, The Netherlands

Abstract—*Thedgonia ligustrina*, the agent of *Ligustrum* leaf-spot, and *Gloeosporidiella turgida*, known as a parasite of *Fraxinus*, are reported for the first time on *Syringa* spp. Both fungi were found in Sweden, the first in a tree nursery in the south, and the second on plants cultivated in Uppsala. Brief descriptions and illustration are provided and the distribution of *T. ligustrina* is reviewed.

Key words—anamorph, Ascomycota, Lithuania, taxonomy

Introduction

Several species of *Syringa* (lilac) are cultivated in Sweden for ornamental purposes (Nitzelius 1964). One parasitic fungus found on *S. vulgaris* L., presented for identification to the first author in 1985, and another one found on *S. ×chinensis* Willd. in 2003-2005 by V.M. and O.C., were identified as parasites of *Ligustrum* and *Fraxinus*, respectively, all members of the *Oleaceae*. These fungi were also isolated in culture, using the procedure described by Constantinescu (1988). Because these fungi have been described and illustrated in detail by previous authors, only short descriptions are provided and few, significant details are illustrated in this paper. The nomenclature of colony colour follows Kornerup & Wanscher (1983), the abbreviation of herbaria those from Holmgren et al. (1990), and UPSC is the acronym of Uppsala University Culture Collection of Fungi.

Thedgonia ligustrina (Boerema) B. Sutton (Figs 1a & 2a)

A leaf-spot disease of *Syringa vulgaris* cultivated in a tree nursery in southern Sweden was observed in 1985. In late autumn the same year several leaves showing symptoms and the presence of a fungus were received for identification at UPSC.

On the upper leaf surface the fungus produces small, 0.5-1.5 mm round to irregular, silvery spots, surrounded by a narrow, reddish brown margin. When the spots coalesce, larger portions of the surrounding tissue become brown, necrotic. On the corresponding lower surface, an ochreous tuft develops composed of conidiophores emerging from colourless stromata and conidia. The conidiophores are agglomerated in fascicles, colourless, 30-75 µm long, 4-6 µm wide, with 1-2 (-3) flat, not thickened scars located at the tip or slightly below. The conidia are colourless, more or less cylindrical, 35-75 × 4-6 µm, 0-4 septate.

Cultures initiated from conidia: on 2% malt-extract agar, the fungus grows very slowly, forming colonies of 3-4 mm diam in 10 days at ca. 20°C; the colonies appear umbonate, Brownish Orange to Light Brown (6CD 4-5), reverse Dark Brown (8F 4-5), margin narrow, deep; exudate and diffusible pigment absent; the surface is velvety and composed of erect hyphae Advancing hyphae are colourless, straight to slightly curved, 3-3.5 µm diam, septate every 15-25 µm, sparingly branched, branches at ca. 50-70°, usually arising just below the septum of the subtending hypha; older hyphae yellowish to brownish, 5-8 µm diam, constricted at septa, later becoming moniliform to chlamydospore-like and then up to 13 µm diam.

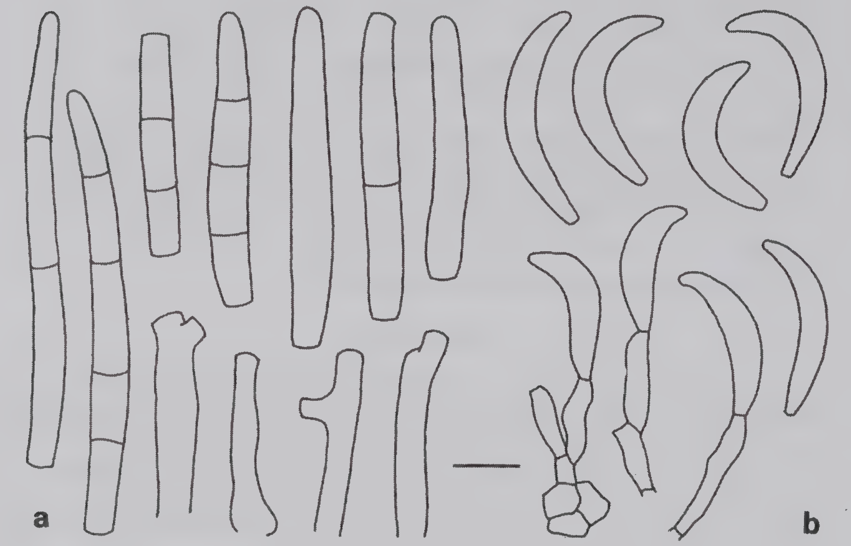


Fig. 1. Fungi on *Syringa*; conidiogenous cells and conidia of **a** - *Thedgonia ligustrina* (*UPS F-117834*) and **b** - *Gloeosporidiella turgida* (*UPS F-118065*). Bar = 10 µm).

SPECIMENS EXAMINED — On *Syringa vulgaris*: **SWEDEN. Skåne,** Veberöd, Björkhaga tree nursery (55°38'N 13°28'E), XI. 1985, coll. L. Nilsson, det. O. Constantinescu *UPS F-117834*; living culture *UPSC 1699* (now at *CBS 113536)*; dried culture *UPS F-118064*. On *Ligustrum vulgare*: **GERMANY. Bayern, Oberbayern,** Bad Reichenhall, 'Thumsee' (47°43'N 12°50'E), 13 VIII 1996, U. Braun in Triebel, Microfungi exsiccati 348 *UPS F-11959*. **KOREA. Suwŏn,** Guundong street, near the entrance to NIAST. (37°15'N 127°00'E), 25 X 2003, V. Mel'nik *LE 214697*. **LITHUANIA. Alytus,** Punia Forest (54°32'N 24°04'E), 7 IX 2000, A. Treigienė *BILAS 27697*; environs of Vilnius, Pašilaičiai (54°44'N 25°42'E), 15 VII 2002, A. Treigienė *BILAS 27698*. **ROMANIA.** București, Mogoșoaia Castle park (44°31'N 26°00'E), 26 X 1968, O. Constantinescu in Herb. Mycol. Romanicum 2010 *BUCM*; *UPS*; living culture *CBS 547.71*.

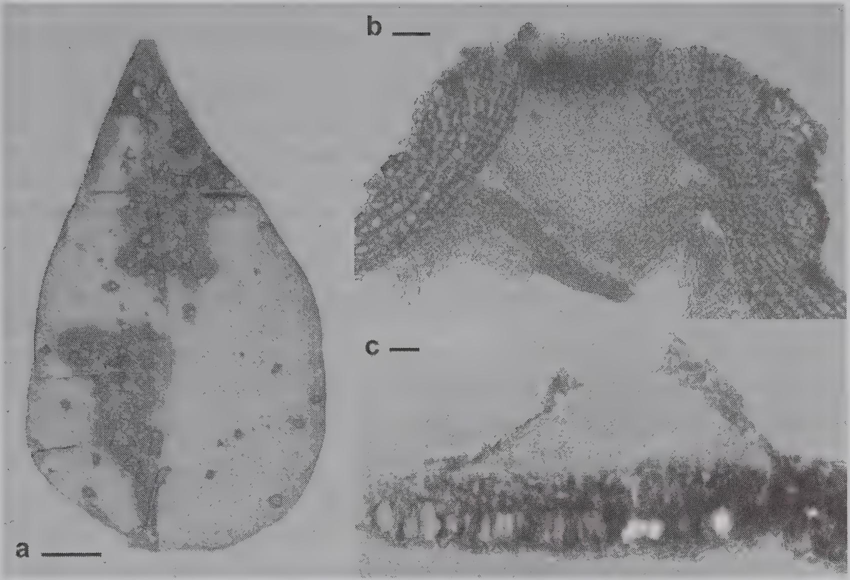


Fig 2. **a** - Leaf spot of *Syringa vulgaris* produced by *Thedgonia ligustrina* (*UPS F- 117834*). **b** - Stromatic conidioma of *Gloeosporidiella turgida* on twigs of *Syringa ×chinensis* (*UPS F-118065*). **c** - Acervular conidioma of *Gloeosporidiella ribis* on leaves of *Ribes rubrum* (*UPS F-120164*). Bars = 1 cm in **a**, 75 µm in **b**, and 25 µm in **c**.

Discussion — The characters of the fungus present on *Syringa* agree well with the detailed descriptions and illustrations of *Thedgonia ligustrina* from *Ligustrum* in Sutton (1973), Yoshikawa & Yokoyama (1992), and Braun (1995). Our specimen was also compared with several herbarium specimens.

Thedgonia ligustrina was recorded previously only on various species of *Ligustrum*, such as *L. japonicum* Thunb., *L. obtusifolium* Siebold & Zucc., *L. ovalifolium* Hassk., and *L. vulgare* L. *Syringa* is thus a new host record. Both *Ligustrum* and *Syringa* belong to the family *Oleaceae*. The presence of *T. ligustrina* on *Syringa*, particularly in a tree nursery, seems to be the result of a 'host jump' in the sense of Parlevliet (1979).

With the recent records by Stakvilevičienė (2004) and Mel'nik et al. (2005), the distribution of *T. ligustrina* includes Austria, Denmark, France, Holland, Germany, Japan, Lithuania, Korea, Romania, Sweden, United Kingdom and USA.

Attempts by Yoshikawa & Yokoyama (1992) to isolate this fungus failed. *Thedgonia ligustrina* has been tested as a possible agent of biological control of *Ligustrum* in La Réunion (http://www.cabi-bioscience.org/Html/activitiesDevelopinCountries.htm).

Gloeosporidiella turgida (Berk. & Broome) B. Sutton (Figs 1b & 2b)

The fungus was found on dry, dead, ca. 2-3 mm thick twigs of *Syringa ×chinensis*. It appears as 0.2-0.5 mm pustules emerging through the bark, appearing ochre, later becoming brownish. Each conidioma is seated on a basal stroma, ca. 200 µm wide × ca. 150-200 µm high; conidiophores are colourless, simple or sparingly branched, conidiogenous cells are 10-20 µm long, 3-4 µm broad; conidia are colourless, curved, falcate to sickle-shaped, 22-33 × 4-5 µm, narrowing to the rounded tip and the more or less truncate base.

Colonies initiated from conidia: on 2% malt-extract agar slow growing, attaining a diam of 5-7 mm in 10 days and 12-13 mm after 25 days at ca. 20°C, appearing flat, centrally umbonate, surface lanose, Brownish Orange (7C 3-4) to Reddish Grey, to Brownish Grey (8B, C 2); reverse vivid Brownish Orange (6C 8; 7C 8), margin narrow, entire in young colonies but fimbriate in older ones, exudate absent, diffusible pigment Brownish Orange. Advancing hyphae colourless, wavy to geniculate-like, ca. 2-3 µm diam, sparingly and irregularly branched at ca. 35-40° angle, septate every 18-22 µm, older hyphae yellowish and slightly wider. Spherical, 0.5-1 mm diam sclerotium-like bodies, composed of interwoven hyphae are formed in fresh cultures. These may represent young stages of conidiomata but they did not develop further after 6 months. These sclerotial bodies are not formed in later subcultures.

> **SPECIMENS EXAMINATED**: On *Syringa* ×*chinensis*: **SWEDEN**, **Uppland**, Uppsala, Källparksgatan 12 (59°53'N 17°39'E), 22 VI 2003, V. Mel'nik *LE 214424; UPS F-118065*; dried culture *UPS F-118066*; living culture *CBS* 116473; ditto, 15 IX 2004, O. Constantinescu *UPS F*-118067; ditto, 15 VI 2005 *UPS F-120165*. On *Fraxinus excelsior*: **LITHUANIA, Lazdijai Distr.**, environs of Gerdašiai (53°57'N 23°54'E), 25 VII 1995, A. Treigienė *BILAS 27694*; **Kėdainiai Distr.**, Berunkiškiai Forest (55°13'N 24°08'E), 18 VI 1999, A. Treigienė *BILAS 27695*. ditto, Stebuliai Forest (55°19'N 24°06'E), 31 V 2000, A. Treigienė *BILAS 27696*.

Discussion — The characters of the fungus on *Syringa* agree well with the description and illustration of *Gloeosporidiella turgida* provided by Pirozynski & Morgan-Jones (1968) under *Cryptosporiopsis turgida* (Berk. & Broome) Piroz. & Morgan-Jones. *Gloeosporidiella turgida* is a known parasite of *Fraxinus* and was recorded from United Kingdom (Pirozynski & Morgan-Jones 1968). It was also found in Lithuania (A. Treigienė *in litt.*). This fungus was indicated as the agent responsible for canker of ash in the USA (Adams 2001) but, according to M. Putnam (*in litt.*), a more thorough investigation revealed that the fungus involved was *Phlyctema vagabunda* Desm. (Rossman et al. 2002). *Syringa* is a new host record for *G. turgida*. As in the case of *Thedgonia ligustrina*, the new host belongs to the same family.

Gloeosporidiella turgida is based on *Cryptosporium turgidum* Berk. & Broome. The placement in *Gloeosporidiella* was accepted by Verkley (1999) in his monograph of *Pezicula*. However, there are important morphological discrepancies between *C. scutellata* (G.H. Otth) Petr., the type species of *Cryptosporiopsis* and anamorph of *Pezicula ocellata* (Pers. : Fr.) Seaver (syn. *Ocellaria ocellata* (Pers. : Fr.) J. Schröt.), and *G. ribis* (Lib.) Petr., the type species of *Gloeosporidiella* and anamorph of *Drepanopeziza ribis* (Kleb.) Höhn. In the former the conidioma is stromatic whereas in the latter it is acervular, subcuticular and lacks stroma (Figs 2b,c). Consequently, *G. turgida* seems to be better accommodated in *Cryptosporiopsis*, but it should be noted that its teleomorph is unknown. Other *Cryptosporiopsis* anamorphs are associated with two phylogenetically distinct teleomorph genera, *Pezicula* and *Neofabraea* p.p., and also closely related to *Phlyctema* (teleomorph *Neofabraea alba* (E.J. Guthrie) Verkley), and *Foveostroma* anamorphs of *Dermea* (Abeln et al. 2000).

Acknowledgements

We are indebted to Lennart Nilsson for the specimen of *Syringa vulgaris*, to Aušra Treigienė for information on the presence of *G. turgida* in Lithuania, to Uwe Braun for providing a copy of the text regarding *Thedgonia* from his monograph, to Melodie Putnam for drawing attention to the paper by Rossman et al. (2002), to Svengunnar Ryman for information about the presence of *Syringa* spp. in Sweden, and to Directors and Curators of BILAS, BUCM and UPS herbaria for permission to examine specimens in their keeping. Special thanks are due to Amy Rossman, Shaun Pennycook and Uwe Braun for their criticism as pre-submission reviewers.

Literature Cited

Abeln ECA, Pagter MA de, Verkley GJM 2000. Phylogeny of *Pezicula*, *Dermea*, and *Neofabraea* inferred from partial sequences of the nuclear ribosomal RNA gene cluster. Mycologia 94: 685-693.

Adams G. 2001. A new disease of ash causing economic losses in Michigan In: Nursery and Landscape Research. Projects and Education Programs (Brown-Rytlewski D., Ed.), p. 29. Michigan State University.

Braun U. 1995. A monograph of *Cercosporella, Ramularia* and allied genera *(*phytopathogenic hyphomycetes*)*. 1. IHW-Verlag, Eching bei München.

Constantinescu O. 1988. An instrument and procedure for single-spore isolation. Trans. Brit. Mycol. Soc. 91: 700-702.

Holmgren PK, Holmgren NH, Barnett LC [Eds] 1990. Index Herbariorum. Part I: The Herbaria of the World. 8th ed. New York Botanical Garden, Bronx.

Kornerup A, Wanscher JH. 1983. Methuen Handbook of Colour. Methuen, London.

Mel'nik VA, Shin HD, Jee HJ, Cho WD. 2005. Materialy k izuchennyu raznoobraziya gribov Korei [Contribution to the study of fungal diversity in Korea]. Mikol. Fitopatol. 39(3): 61-68. In Russian.

Nitzelius T. 1964. Blommande buskar och träd [Flowering bushes and trees]. Saxon & Lindströms, Stockholm. In Swedish.

Parlevliet JE. 1979. The co-evolution of host-parasite systems. Symb. Bot. Upsal. 22 (4): 39-45.

Pirozynski KA, Morgan-Jones G. 1968. Notes on microfungi. III. Trans. Brit. Mycol. Soc. 51: 185-206.

Rossman AY, Castlebury LA, Adams GC, Putnam ML. 2002. *Phlyctema vagabunda* isolated from coin canker of ash trees in Michigan. Pl. Dis. 86: 442.

Stakvilevičienė S. 2004 *Thedgonia ligustrina* (Boerema) B. Sutton - naujas paprastojo ligustro *(Ligustrum vulgare)* L. dėmėtligės sukėlėjas Lietuvoje [*Thedgonia ligustrina*. a new agent of brown leaf spot of *Ligustrum vulgare* in Lithuania]. Dendrologia Lithuaniae 7: 123-127. In Lithuanian.

Sutton BC. 1973. Some hyphomycetes with holoblastic sympodial conidiogenous cells. Trans. Brit. Mycol. Soc. 61: 417-429.

Verkley GJM. 1999. A monograph of the genus *Pezicula* and its anamorphs. Stud. Mycol. 44: 1-180.

Yoshikawa M, Yokoyama T. 1992. *Thedgonia ligustrina* on *Ligustrum japonicum* and *Cercospora kyotensis* sp. nov. on *Hydrangea serrata* var. *thunbergii*. Trans. Mycol. Soc. Japan 33: 177-184.

MYCOTAXON

Volume 94, pp. 181–187 October–December 2005

Further notes on the molecular taxonomy of *Metarhizium*

Bo Huang[1, 2], Richard A. Humber[3], Shigui Li[4],
Zengzhi Li[*1] & Kathie T. Hodge[2]

zzli@ahau.edu.cn
[1] Anhui Provincial Key Laboratory for Microbial Pest Control,
Anhui Agricultural University, Hefei 230036, China

[2]Department of Plant Pathology, Cornell University
Ithaca, NY 14853, USA

[3] USDA Plant, Soil & Nutrition Laboratory, Tower Road
Ithaca, NY 14853, USA

[4] Institute of Soil and Fertilizer Sciences
Chinese Academy of Agricultural Sciences, Beijing 100081, China

Abstract—The ITS1-5.8S-ITS2 rDNA regions from *Metarhizium guizhouense, M. pingshaense, M. iadini,* and four other *Metarhizium* isolates were amplified and sequenced. Bayesian analyses showed that *M. pingshaense* should be treated as synonym of *M. anisopliae* var. *anisopliae*; that *M. guizhouense* should be treated as a synonym of *M. anisopliae* var. *anisopliae*; and that *M. iadini* is a later synonym of *M. flavoviride* var. *pemphigi*. Several previously unidentified isolates, RCEF0898, ACCC30130 and RCEF1259, the taxonomic positions of which have been unclear or suspicious, were identified as *M. anisopliae* var. *anisopliae, M. anisopliae* var. *anisopliae* and *M. flavoviride* var. *pemphigi,* respectively.

Key words—entomopathogenic fungi, *Clavicipitaceae,* synonymy

Introduction

Under natural conditions, fungi are frequent and often important natural mortality factors in insect populations (Milner, 2000). Entomopathogenic fungi may exhibit very narrow or very broad host ranges, depending on the species and isolate. *Metarhizium anisopliae* var. *anisopliae* (abbreviation: *M.a. anisopliae*) has one of the broadest host ranges, having been found to naturally infect over 200 insect species (Milner, 2000). Because of the absence of detrimental environmental effects, lack of residues in meat or crops, and ease of mass production, some species of *Metarhizium* can be used for the control of many insect pests as an environmentally acceptable alternative to chemical insecticides. Some *Metarhizium* mycoinsecticides are now registered in Australia, Europe and the USA as well as being used in countries such as Brazil and China, and have played important roles in controlling agricultural and forest insect pests.

---

*Author for correspondence

Two *Metarhizium* species and two varieties were accepted by Tulloch (1976) in her important review. Most recently, 3 species and 4 varieties have been accepted (Rombach et al. 1986) based on morphological data. Molecular phylogenetic studies of large and small subunit rDNA by Liu et al. (1994) addressed some Chinese *Metarhizium* strains. Later, Driver et al. (2000) proposed many new varieties based on their study of ITS sequences. Information from additional genetic loci and additional isolates is needed to fully resolve *Metarhizium* taxonomy.

The status of five *Metarhizium* species described from China has been unclear, largely because cultures were difficult to obtain. These are *Metarhizium cylindrosporae* Q.T. Chen & H.L. Guo, *M. guizhouense*, *M. pingshaense*, *M. taii*, and *M. iadini*. Recently Huang et al. (2004) showed that *M. cylindrosporae* is correctly placed in *Metarhizium*, rather than in *Nomurea* as proposed by Tzean et al. (1993), and that *Nomuraea viridula* Tzean et al. also belongs in *Metarhizium*. Huang et al. (2005) proposed that *M. taii* should be considered a synonym of *M.a. anisopliae* and revealed that *Cordyceps taii* Z.Q. Liang & A.Y. Liu is the teleomorph of *M.a. anisopliae*. This study deals with the taxonomy and identity of the remaining three species based on *ex type* cultures. The identity of several interesting *Metarhizium* isolates is also addressed. ITS sequences were used as molecular markers to address these problems.

Materials and Methods

Fungal isolates and culture—Four isolates of *Metarhizium* deposited at the Institute of Soil and Fertilizer Sciences, Chinese Academy of Agricultural Science (ACCC) were provided by Li Sigui, the others were conserved in the Anhui Provincial Key Laboratory for Microbial Pest Control, Anhui Agricultural University (RCEF). Table 1 lists the hosts, identifications, and collecting locations of studied isolates. Those isolates that are included in this study only by means of sequence data obtained from GenBank are shown in Figure 1.

Table 1. Original identification, insect hosts, and geographic origins of six isolates of *Metarhizium* spp. studied here.

GenBank Accession	Strain No.	Original Identification	Host	Location
DQ177434	RCEF0898	*M. anisopliae*	Soil	Anhui
DQ177428	ACCC30105 (ex type)	*M. pingshaense*	*Alissonotum* sp. / Coleopt.	Guangdong
DQ177430	ACCC30115 (ex type)	*M. guizhouense*	*Hepialus* sp. / Lepidopt.	Guizhou
DQ177429	ACCC30124 (ex type)	*M. iadini*	*Rhynchites coreanus* / Coleopt.	Guizhou
DQ177432	ACCC30130	*M. flavoviride* var. *minus*	Uncertain	Uncertain
DQ177435	RCEF1259	*Metarhizium* sp.	Larva of Lepidoptera	Anhui

For DNA extraction, spores or mycelium were transferred to a Petri dish containing potato dextrose agar medium overlaid with a disk of autoclaved cellophane. The Petri dish was sealed with Parafilm, and placed in an incubator at 25°C for about 1 week. Mycelium was then scraped from the cellophane, then stored at -20°C prior to DNA extraction.

DNA extraction, amplification and sequencing—Total genomic DNA was extracted from frozen mycelium by using a benzyl chloride method (Zhu et al. 1994). DNA pellets were air-dried and resuspended in 200 μl sterilized TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0). The quantity and quality of total genomic DNA was observed on a 0.8% TBE agarose gel stained with ethidium bromide. DNA for sequencing was amplified enzymatically using the polymerase chain reaction. The internal transcribed spacer regions (ITS1 and ITS2) of the ribosomal DNA operon, including the 5.8S gene, were amplified using primers ITS4 and ITS5 (White et al. 1990). The PCR reaction was performed with 0.5 μl of the dissolved total DNA in a 50 μl reaction volume containing 200 μM of each dNTP, 2 mM $MgCl_2$, 0.1 mM of each primer, and 2 units *Taq* DNA polymerase with PCR buffer (Sangon, China). The PCR reactions were placed in a thermal cycler (Techine, UK) under the following temperature-cycling parameters: Step 1) 5 min at 95°C; Step 2) 35 cycles of 1 min at 94°C, followed by 1 min at 54°C, and 1 min at 72°C; Step 3) 10 min at 72°C. The resulting products were examined on a 1.2 % TBE agarose gel stained with ethidium bromide. PCR fragments were purified using the Wizard™ PCR Preps DNA Purification System Kit (Promega Co., France). PCR products were sequenced using the above-mentioned primers on an ABI 3700 automated sequencer at Shanghai Genecore Biotechnologies Company. Products were sequenced in both 5' to 3' and 3' to 5' directions.

Phylogenetic analyses—DNA sequences generated by us and downloaded from GenBank (see Table 1 in Huang et al. 2005) were aligned using Clustal X 1.81 (Thompson et al. 1997), and the alignment was refined by eye. *Beauveria bassiana* (Bals.-Criv.) Vuill., a related clavicipitaceous anamorph, was used as the outgroup. Parsimony analysis performed in PAUP* version b10 (Swofford 2002) using a heuristic search, but it failed to complete using available memory, apparently because of a high number of equally parsimonious trees arising from the similar sequences among isolates of *M.a. anisopliae*. Bayesian analysis was carried out using MrBayes 3.0 b4 (Huelsenbeck 2000, Huelsenbeck et al. 2001). We used a 6 parameter model to run four chains for 500,000 generations, sampling every 100 generations. The first 500 trees were discarded (burn in), and the remaining trees were saved to a file. A 50% majority rule consensus tree (Fig. 1) was then calculated using PAUP*.

Results and Discussion

Metarhizium pingshaense* and *Metarhizium guizhouense—*M. pingshaense* is mainly distinguished from *M. anisopliae* var. *anisopliae* in the color of the colony and in that the spore chains are formed of individual spores connected eccentrically (Guo et al. 1986). Phylogenetic analysis reveals that the *ex type* isolate of *M. pingshaense* belongs to a clade that includes 17 isolates of *M.a. anisopliae,* including the *ex neotype* isolate FI1029. *M. pingshaense* differs from the *ex neotype* isolate by three base pairs in the ITS

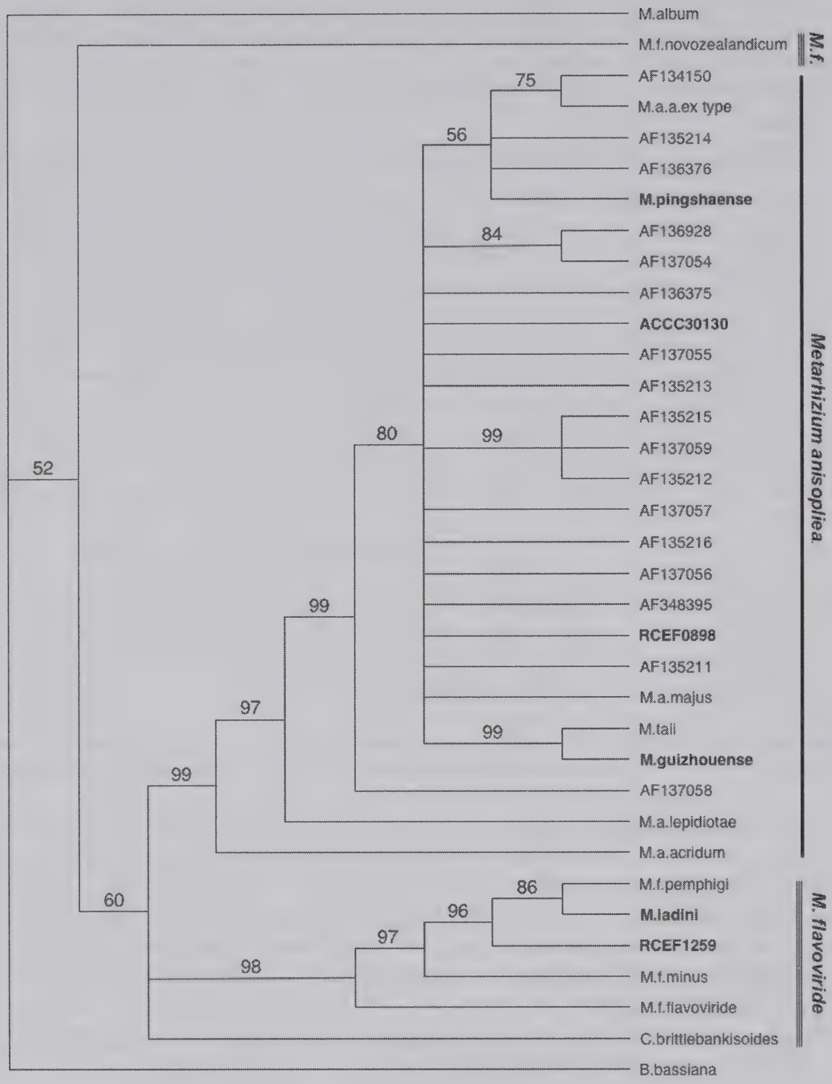

M.album
M.f.novozealandicum
M.f.
75
AF134150
M.a.a.ex type
56
AF135214
AF136376
M.pingshaense
84
AF136928
AF137054
AF136375
ACCC30130
AF137055
AF135213
AF135215
80
99
AF137059
52
AF135212
AF137057
AF135216
AF137056
99
AF348395
RCEF0898
AF135211
97
M.a.majus
99
M.taii
99
M.guizhouense
AF137058
M.a.lepidiotae
M.a.acridum
Metarhizium anisopliea
86
M.f.pemphigi
60
96
M.ladini
97
RCEF1259
98
M.f.minus
M.f.flavoviride
C.brittlebankisoides
B.bassiana
M. flavoviride

Figure 1. Bayesian phylogeny of *Metarhizium* spp. based on the analysis of ITS sequence data. Posterior probabilities (>50%) are given as percentages above the branches. (*M.f.* = *M. flavoviride*, and the six isolates in this study are indicated in bold face.)

sequence. We therefore propose that *M. pingshaense* should be regarded as a synonym of *M.a. anisopliae.* The conclusion is supported by the earlier conclusion of Liu et al. (1994) based on identical partial sequences from small (18S) and large (28S) subunit rRNAs. Negligible differences in serology among *M. pingshaense* and several strains of *M.a. anisopliae* (Hu et al. 1996) provide further support. There can be no question from the integrative data here that *M. pingshaense* cannot be maintained as an independent species, and it should be treated as a synonym of *M.a. anisopliae*.

The *ex type* isolate of *M. guizhouense* also fell in the clade including all isolates of *M.a. anisopliae* except isolate FI379 (Fig. 1). Compared with the *ex neotype* isolate FI1029, *M. guizhouense* differed in 6 base positions in the ITS regions. Two per cent divergence in the ITS between *M. guizhouense* and isolate FI1029 falls within the scope of variation among *M.a. anisopliae* isolates (0-2.3%). According to Liu et al. (1986), conidial length in *M. guizhouense* measured 6.7-7.3 μm, while Driver et al. (2001) gave 5–8.0 μm as the range for conidial length in *M.a. anisopliae*.

Liu et al. (2001) suggested that *M. taii* and *M. guizhouense* were the same fungus based on their morphology and type of esterase isoenzyme. The ITS sequences of these two species are similar, differing in only two positions. Both species attack the larvae of Lepidoptera. Huang et al. (2005) have demonstrated that *M. taii* must be treated as a synonym of *M.a. anisopliae* based on its morphology and ITS sequence. We also consider that *Metarhizium guizhouense* is a synonym of *M.a. anisopliae*, based on morphological characters, ITS sequence and the conspecificity of *M. taii* and *M. guizhouense*.

Metarhizium anisopliae (Metsch.) Sorokīn var. *anisopliae*, [Plant Parasites of Man and Animals as Causes of Infectious Diseases] 2: 267 (1883) [in Russian].

Synonym: *Metarhizium pingshaense* Q.T. Chen & H.L. Guo, Acta Mycol. Sinica 5: 179 (1986) [in Chinese].

Synonym: *Metarhizium guizhouense* Q.T. Chen & H.L. Guo, Acta Mycol. Sinica 5: 181 (1986) [in Chinese].

Synonym: *Metarhizium taii* Z.Q. Liang & A.Y. Liu, Acta Mycol. Sinica 10: 260 (1991) [in Chinese].

***Metarhizium iadini* and identification of isolate RCEF1259**—According to Guo (1991), *M. iadini* is differentiated by its light green colonies in culture, its ovoid conidia with light plain base, (4-)5.7(-6)×(1.8-)2.2(-2.4)μm, and its coleopteran host, *Rhynchites coreanus*. *Metarhizium flavoviride* var. *pemphigi* (abbreviation: *M.f. pemphigi*) is similar, with ovoid to elongate conidia measuring 5.4×2.4 μm, a light green conidial mass, and a root aphid host. In our analysis (Fig. 1) the *ex type* isolate of *M. iadini*, *M.f. pemphigi* and strain RCEF1259 grouped together in a strongly supported clade. Among them there are 8 nucleotide differences in the ITS regions. *Metarhizium iadini* and *M.f. pemphigi* were distinguished by only small differences in host and growth rate at low temperature (the former reaches 15 mm, and the latter 40 mm at 10°C after 3 weeks). However, they share other characteristics such as conidial shape and size, and color of colony. Also, these two species display similar sequences in the ITS region. We conclude that *M. iadini* and *M.f. pemphigi* are conspecific. The former name is legitimate, but the lack of a Latin diagnosis makes it invalidly published (Art. 42). Accordingly, the isolate ACCC30124, originally identified as *M. iadini*, is properly identified as *M.f. pemphigi*.

Strain RCEF1259 was isolated from an entomogenous fungus specimen collected from Anhui Province in China. A synnemata formed on a Lepidoptera larva is 5 cm long, and green at the apex. Conidial size and shape are similar to those of *M.f. pemphigi*. This isolate formed a few light green conidia, and a floccular mycelium on PDA after 10 days. Because synnemata are unusual in *Metarhizium*, the isolate was included in this study to assess its relationships and identification. Compared to *M.f. pemphigi*, RCEF1259 differs in 4 positions in ITS region, and also shows 3 deletions. We conclude that it should be recognized as *M.f. pemphigi* based on morphological and molecular data.

Before the current study, only two isolates of *M.f. pemphigi* had been recorded. Similarities among these newly recorded isolates provide evidence that it is reasonable for *M.f. pemphigi* to be recognized as a distinct variety. The light green color of the conidial masses is apparently an important identifying morphological character for this variety.

Metarhizium flavoviride var. *pemphigi* Driver & Milner, Mycol Res: 104: 144 (2000).
Synonym: *Metarhizium iadini* H.L. Guo, The Study and Application of *Metarhizium*: 27 (1991) [in Chinese].

Identification of isolates ACCC30130 and RCEF0898—Isolate ACCC30130 was identified as *M. flavoviride* var. *minus* in a catalogue of Chinese agricultural isolates (Guo & Ning, 1991). According to Liu et al.'s 1994 study, *M. pingshaense*, ACCC30130 and *M.a. anisopliae* shared similar 18S and 28S DNA sequences. However, the present study based on morphology and ITS sequences suggests that ACCC30130 should be attributed to *M.a. anisopliae*. The strain differs from the *M.a. anisopliae ex neotype* isolate by only 3 positions in the ITS regions.

The strain RCEF0898 differs by 6 nucleotides from the neotype strain of *M.a. anisopliae*; it falls into the same clade as most isolates of the latter (Fig. 1). The morphology of RCEF0898 is similar to *M.a. anisopliae* except that some large conidia measured up to 9×3.5 µm. Although *Metarhizium taii* has been reported to produce some large conidia, it was synonymized with *M.a. anisopliae* in a recent study (Huang et al. 2005). This small difference in conidial length is not considered taxonomically significant. Therefore, strain RCEF0898 is also identified as *M.a. anisopliae.*

Acknowledgements

We are grateful to Drs. Wenying Zhuang and Yijian Yao for reviewing the manuscript. This project was supported partly by the National Natural Science Foundation of China (Grant No. 30300004), the Key Laboratory of Systematic Mycology and Lichenology (Grant No. 912), the Anhui Provincial Science Foundation for Excellent Youth and program for "NCET".

Literature Cited

Driver F, Milner RJ, Trueman WH. 2000. A taxonomic revision of *Metarhizium* based on a phylogenetic analysis of rDNA sequence data. Mycol Res 104: 134-150.

Guo HL. 1991. The study and application of *Metarhizium*. Chinese Agriculture Press, Beijing.

Guo HL, Ye BL, Yue YY, Chen QT, Fu CS. 1986. Three new species of *Metarhizium*. Acta Mycol Sinica 5: 177-184.

Guo HL, Ning GZ. 1991. Catalogue of agricultural isolates from China. China Agricultural Sciences and Technology Publishing House, Beijing.

Liang ZQ, Liu AY, Liu JL. 1991. A new species of the genus *Cordyceps* and its *Metarhizium* anamorph. Acta Mycol Sinica 10: 257-262.

Liu ZW, Guo HL, Kurtzman CP. 1994. Phylogeny of *Metarhizium* species as determined by partial ribosomal RNA sequences. Acta Mycol Sinica 13: 139-151

Liu ZY, Liang ZQ, Whalley AJS, Yao YJ, Liu AY. 2001. *Cordyceps brittlebankisoides,* a new pathogen of grubs and its anamorph, *Metarhizium anisopliae* var. *majus.* J Invert Pathol 78, 178-182.

Hu JJ, Fan MZ, 1996. The study on the immunological characters of the genus *Metarhizium*. Journal of Anhui Agricultural University 23 (3): 433-437.

Huang B, Li SG, Li CR, Fan MZ & Li ZZ. 2004. Studies on the taxonomic status of *Metarhizium cylindrospora* and *Nomuraea viridula*. Mycosystema 23: 33-37.

Huang B, Li CR, Humber RA, Hodge, KT, Fan MZ & Li ZZ. 2005 (2006). Molecular evidence for the taxonomic status of *Metarhizium taii* and its teleomorph, *Cordyceps taii* (*Hypocreales, Clavicipitaceae*). Mycotaxon 94: 137–147.

Huelsenbeck JP. 2000. MrBayes: Bayesian inference of phylogeny (software). New York: University of Rochester.

Huelsenbeck JP, Ronquist F, Nielsen ES, Bollback JP. 2001. Bayesian inference of phylogeny and its impact on evolutionary biology. Science 294: 2310-2314.

Milner, RJ. 2000. Current status of *Metarhizium* as a mycoinsecticide in Australia. Biocontrol News and Information 20: 47-50.

Rombach MC, Humber RA, Roberts DW. 1986. *Metarhizium flavoviride* var. *minus* var. nov., a pathogen of plant- and leafhoppers on rice in the Philippines and Solomon Islands. Mycotaxon 27: 87-92.

Swofford D. 2002. PAUP*: Phylogenetic analysis using parsimony (*and other methods), version 4. Sunderland, Massachusetts: Sinauer Associates.

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG. 1997. The Clustal X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nuc Acids Res 24: 4876-4882.

Tulloch M.1976. The genus *Metarhizium*. Trans Brit Mycol Soc 66: 407-411.

White TJ, Bruns T, Lee S, Taylor J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ, eds. PCR Protocols: A Guide to Methods and Applications. San Diego, California: Academic Press Inc. pp 315-322.

Zhu H, Qu F, Zhu LH. 1994. Isolation of genomic DNAs from fungi using benzyl chloride. Acta Mycol Sinica 13: 41-47.

MYCOTAXON

Volume 94, pp. 189–214 October–December 2005

The genus *Hymenochaete* (Basidiomycota, Hymenomycetes) in the Hawaiian Islands

Erast Parmasto

erast.parmasto@zbi.ee
Institute of Agricultural & Environmental Sciences, Estonian University of Life Sciences
181 Riia St., 51014 Tartu, Estonia

Robert L. Gilbertson

rlg@ag.arizona.edu
Department of Plant Sciences, Division of PlantPathology, University of Arizona
Tucson, AZ 85721, USA

Abstract — Eighteen species of the genus *Hymenochaete* found in the Hawaiian Islands are described, including new species *H. flexosetosa, H. geniculata* and *H. legeri. H. attenuata* sensu G. Cunn., found in New Zealand, is different from *H. attenuata* (Lév.) Lév.; it is described as a new species *H. nothofagicola*. Main source of the origin of Hawaiian *Hymenochaete* biota seems to be Australasia.

Key words — New Zealand, distribution, spore variation

Introduction

In his monograph of *Hymenochaete,* Burt (1918) did not mention any collections from the Hawaiian Islands nor did Cunningham (1957) in his paper on New Zealand and Australian 'Thelephoraceae" or Léger (1998) in his world monograph of the genus *Hymenochaete*. Burt (1923) in a paper on higher fungi of the Hawaiian Islands mentioned three species: *H. tenuissima* Berk. (a synonym of *H. rheicolor)* was collected "on decaying wood, F.L. Stevens, 118, 967", but the specimens were not found by us in BPI or NY; *H. cinnamomea* (Pers.) Bres. and *H. spreta* Peck have been misidentified by Burt (see below under *H. semistupposa* and *H. tomentelloidea*). Gilbertson & Adaskaveg (1993) mentioned *H. anomala* and *H. corrugata* collected by them in 1990. Gilbertson & Hemmes (1997) described a new species, *H. tomentelloidea* from South Hilo District; Gilbertson et al. (2002) listed four species of *Hymenochaete (H. anomala, H. corrugata, H. mougeotii* and *H. tomentelloidea)* collected in Hawai'i, Kaua'i and Maui Islands in their check list of wood-rotting Basidiomycetes. Apparently no other data on Hawaiian *Hymenochaete* have been published.

Materials and methods

In the National Fungus Collections (BPI), there are specimens of eight *Hymenochaete* species, mainly previously unidentified, collected by C.L. Shear and N.E. Stevens from Hawai'i. In the Herbarium of the New York Botanical Garden (NY), one specimen of *H. attenuata* collected by J. Kliejunas was found. Collections by R.L. Gilbertson, also by J.E. Adaskaveg and D.E. Hemmes from 1990 to 2002 contain 114 specimens of *Hymenochaete* reported in this paper. Collections are deposited in ARIZ and their duplicates in TAA. For comparison, some specimens collected by S.H. Wu in Taiwan and by the senior author in India (both in TAA) have also been examined.

Authors of fungal names follow Kirk & Ansell (1992). Herbarium acronyms are after Holmgren, Holmgren & Barnett (1990). Names of (phyto)geographical regions are given according to the Plant Taxonomic Database Standard No. 2, Edition 2 (Brummitt, 2001). Colour of the basidiomata of *Hymenochaete* species is very changeable during their development, and most descriptions published until now are saturated with different colour terms. To simplify the descriptions, colour names are given as used in "A mycological colour chart" by Rainer (1970). In parentheses, the additional and more precise colour notations are given according to Munsell, 1976 (M) and Kornerup & Wanscher, 1973 (K & W). In this genus, it is difficult to study spores due to their small measurements and Brownian movement when an ordinary microscope is used. That is why measurements and figures of spores were made with the aid of a Sony CCD Video Camera attached to a Nikon Labophot 2 microscope and analyzed by Global Lab Image (Data Translation Inc.) software. All descriptions given below are based on Hawaiian specimens; when these differ significantly from the descriptions published for other regions, the differences are indicated.

Results and discussion

18 species of the genus *Hymenochaete* have been found in Hawaiian Islands. Only one of these *(H. tomentelloidea)* was definitely known until this study. The propagules of these fungi are small and easily transportable by winds (basidiospore length of most Hawaiian species is in the limits of 2.5–8 μm). Nevertheless, the number of species is rather low. For comparison, of the more than 120 species known, 32 have been found in New Zealand. The Hawaiian Islands have an isolated position far from mainlands. Their age – about 0.5–5 mln years (Carson & Clague, 1995) – is perhaps rather short time for invasion of *Hymenochaete* species from Americas, Asia or Australasia. Only two or three species are of pantropical distribution *(H. attenuata, H. unicolor* and possibly also *H. legeri).* Three species are common with South or Central America *(H. berteroi, H. cervina, H. minuscula),* but these have also been found in Australasia. Six Hawaiian species are common with New Zealand (*H. dissimilis, H. innexa, H. semistupposa, H. separata, H. unicolor, H. vaginata*). However, there are almost no data on distribution of *Hymenochaete* species in Papuasia and Southwestern Pacific, i.e., in the regions between Australasia and Hawaiian Islands. One species *(H. muroiana)* has been found in Eastern Asia (Japan, China) and Malesia, and one *(H. separabilis)* in Tropical Africa. According to the currently known distribution, the main source of the origin of Hawaiian *Hymenochaete* biota seems to be Australasia.

The interval of time up to five million years was long enough to cause intensive speciation of plants and insects; about 45% of mosses and 42% of angiosperms are endemic (Eldredge & Evenhuis, 2003). Speciation is possibly more slow in fungi than in other groups of living beings. In Hawai'i, the percentage of endemic fungi (lichenized species excluded) is about 13 (Eldredge & Evenhuis, 2003). Among *Hymenochaete,* four species are endemic: *H. flexosetosa* (a species possibly closely related to pantropical *H. attenuata* and *H. rheicolor* (Mont.) Lév.), *H. geniculata* (closely related to the New Zealand's *H. stratura* G. Cunn.), *H. subdissimilis* ad int. (if different from *H. dissimilis)* and *H. tomentelloidea* (closely related to *H. separabilis).* All these endemic taxa are closely related to their siblings.

Of the 18 species found, only one is common in the Hawaiian Islands *(H. legeri),* two occur occasionally *(H. attenuata* and *H. semistupposa),* all other species are very rare or found only once.

Taxonomy

Key to Hawaiian *Hymenochaete* species

(Some other species, common in Tropical Asia and Australasia are included)

1. Basidiome pileate or effused-reflexed 2
1. Basidiome effused (resupinate) 6

2. Setae 20–50 x 4–8 μm 3
2. Setae 45–110 x 7–15 μm 5

3. Cortex present; setae 30–50 x 5–8 μm; pileus flexible 4
3. Cortex absent; setae 20–35 x 4–5 μm; pileus brittle .... ***H. adusta*** (Lév.) Har. & Pat.
(Found in Japan, Philippines, Vietnam, Australasia)

4. Numerous brown thick-walled hyphidia in hymenium, 2.5–3.5 μm in diam; basidiome up to 500 μm thick but tomentum thin (up to 150 μm)
H. luteobadia (Fr.: Fr.) Höhn. & Litsch.
(Found in Philippines, Vietnam, Australasia)
4. Thick-walled hyphidia absent; tomentum up to 1000 μm thick, later disappearing and then pileal surface black.......... ***H. villosa*** (Lév.) Bres.
(Pantropical. Figs. 1, *1;* 2, *3)*

5. Part of the setae L-shaped, arising from a short thin setal hypha, 40–70 x 7–12 μm; spores (4.8–)5–5.8(–6.2) x 3-3.5(–3.7) μm .... **5. *H. flexosetosa***
5. All setae "normal", 45–70(–80) x 7–12 μm, spores 3.5–5(–5.5) x 1.7–2.2 μm; hyphae of the context above the hymenium densely compacted in parallel**1. *H. attenuata***

6. Setae in apical part with small thorns or teeth (visible at high magnification) 7
6. Setae without thorns 8

7. Setae 30–45 x 5–7(–8) μm, with few (1–3) low broadly conical teeth or protuberances up to 0.5(–1) μm long.......... **13. *H. separabilis***
7. Setae (35–)40–70 x 5–7 μm, with 5–10 broadly conical teeth 0.8-1.5(-2) μm long
.... **16. *H. tomentelloidea***

8. Brown or brownish hyphidia 2–4 μm in diam with thickened or thick walls present, but never monilioid 9

8. Hyphidia absent, or monilioid brown hyphidia or subhymenial hyphae present 12

9. Hyphidia simple, not dendroid or arbusculoid 10

9. Hyphidia dendroidly branched................................ **6. *H. floridea***

10. Spores broad, (5.7–)6–7 x (4.0–)4.3–5(–5.2) µm; basidiomata thick (up to 500–1100 µm) .... **3. *H. cervina***

10. Spores 1.8–3.2 µm broad; basidiomata very thin or up to 300 µm thick 11

11. Spores cylindrical, 5.5–7(–7.5) x 1.8–2.5 µm; basidiomata 50–100 µm thick... **8. *H. innexa***

11. Spores ellipsoidal, 6.5–9.5 x 2.4–3.2 µm; basidiomata up to 300 µm thick .... **18. *H. vaginata***

12. Many hyphae or hyphidia monilioid, with thickenings on walls; context soft membranaceous, of loosely interwoven hyphae 13

12. Hyphae and hyphidia (when present) not monilioid; context absent or its hyphae densely packed 14

13. Setal hyphae present in context; spores 3.7–4.8 x 2.2–2.7 µm ...... **2. *H. berteroi***

13. Setal hyphae absent; spores 3.2–4.5 x 1.7–2.2 µm .... **12. *H. semistupposa***

14. Setae large, (60–)75–100(–120) x 10–15 µm; spores 6–8 µm long .... **9. *H. legeri***

14. Setae smaller, 30–80 x 4–9 µm; spores 3.5–6.5 µm long 15

15. Numerous conglomerates of crystals 8–50 µm in diam present in the context (setal or hyphal layer) 16

15. Conglomerates of crystals absent, crystals in hymenium sometimes present ... 17

16. Setae 40–60(–65) x 7–10 µm; hyphal layer 30–150 µm thick,indistinctly different from the setal layer; spores 3.7–4.4(–5.0) x 1.8–2.5 µm ..... **15. *H. subdissimilis***

16. Setae smaller, (30–)35–45(–55) x 6–7(–8) µm; hyphal layer absent; spores ellipsoid, 5–6.5 x 3–3.5 µm .... **14. *H. separata***

17. Setae partly angled (geniculate) near the base, narrowly fusoid, (35–)40–60 x 4–7 (–8) µm; setal hyphae (embedded horizontal setae) present .... **7. *H. geniculata***

17. Setae never geniculate; setal hyphae absent 18

18. Setae partly bifurcate at base, with slightly curved tip, (30–)40–50(–60) x (5-)6–8 (–9) µm; spores 4–6 x (2.5–)3–3.5 µm; on bamboo .... **11. *H. muroiana***

18. Setae never with bifurcate base; spores 2.2–3.2 µm broad; on other substrata .. 19

19. Setae straight, (35–)45–80 x 5–9 µm; spores ellipsoid, 4.2–5.4 x 2.5–3.2 µm .... **4. *H. dissimilis***

19. Setae smaller, straight, slightly curved or undulate, (30–)35–60(–65) x 4–7 µm; spores almost subcylindrical 20

20. Spores 4.2–5.2 x 2.2–2.5 µm; hymenium ochraceous or yellowish brown .... **10. *H. minuscula***

20. Spores 3.4–4.8 x 2.2–2.8 µm; hymenium umber with fulvous, Sienna or grayish tint .... **17. *H. unicolor***

Annotated list of species

1. **Hymenochaete attenuata** (Lév.) Lév., Ann. Sci. Nat. Bot. III 5: 152. 1846 **not** sensu G. Cunn., 1957. **Fig. 1, *6*; 2, *9***

Basidiomata annual (?), pileate or seemingly effused-reflexed (then umbonate), closely or loosely agnate, soft, papery-coriaceous and flexible when dry; pilei flabelliform to dimidiate, 0.5–2 cm long, 200–500 µm thick, usually imbricate or in rosettes, confluent (up to 5 cm wide); pileal surface velutinous or tomentose when young or near margin, then densely concentrically sulcate and zonate, coarsely radiately fibrillate (silky-fibrous), dull or shiny, Sienna to Umber or dark Cinnamon (M: 7.5 YR 5/8–7, when old 5 YR 4/6; K & W: 6 D 6–8, when old 6 E 6); margin thin, lobate, sometimes plicate, concolorous with the pileal surface or lighter coloured (when young); hymenium smooth, in very old specimens sometimes with low radial folds, not cracked, dark Sienna to light Umber or dark Cinnamon (M: 7.5 YR 6/8, when old 7.5 YR 5/6; K & W: 6 D 5–6 or 5 C 5).

Tomentum present as a layer of loosely interwoven hyphae or as hyphal tufts, (50)–100–450 µm thick; cortex absent; hyphal layer composed of loosely almost radiately interwoven hyphae in upper part and an indistinctly delimited layer 100–150(–250) µm thick of hyphae more densely arranged in parallel above hymenial layer; setal layer present in older specimens when setae are in two indistinct rows.

Hyphal system monomitic; hyphae with thickened walls (in tomentum thick-walled), brownish, sparsely branched, with septa, in hyphal layer 2.5–3.5 µm, in tomentum 4–5 µm in diam; setae sparsely situated or locally numerous, narrowly conical or slightly fusoid, straight or some with curved tip, bluntly acute, (35–)40–70(–80) x 7–12(–14) µm, emerging 20–45 µm above the hymenium, with a thin hyphal sheath, without incrustation; basidioles with thickened and brownish at base rough (finely encrusted) walls, 18–25 x 3–4.5 µm; basidia 20–25 x 3.5–4 µm, with 4 thin sterigmata; spores short cylindrical with one side slightly concave (suballantoid), 3.5–5(–5.5) x (1.5–) 1.6–2.2 µm.

Hawai'i. J. Kliejunas 18a, det. G.A. Escobar, Kahalu'u Forest Preserve, Kona (NY); RLG 17007, 18112 on *Metrosideros polymorpha,* Mile 18, Saddle Rd. Kaua'i. RLG 21081, 21082, 21123 on *Acacia koa,* Nualolo Trail, Koke'e State Park; RLG 20558, 21063, 21067, 22745A on *Eucalyptus* sp., RLG 22735, 22743 on *Eucalyptus globulus,* Makaha Rd.; RLG 21182, on *Psidium guajava,* Ohelo Berry Flat Trail, Koke'e State Park. Moloka'i. RLG 19253 on *Acacia mearnsii* sp., Kamakou Rd.

Comments. This species belongs to the *H. rheicolor*-group of sibling species; their difference is not always clear, and most of the herbarium specimens including types of all species and their synonyms have very few or no spores.

Bresadola has synonymized *H. attenuata* with *H. rheicolor* in one of his later papers (1915: 301) but the two species are considered distinct by other authors. According to Léger (1998), who has studied the types of both species, the main difference is in size of setae and spores: 45–75 x 6–9(–11) µm and 3.5–4.5(–5.5) x 1.5–2(–2.2) µm in *H. attenuata,* 60–105(–120) x (8–)9–12(–13) µm and 5–6 x 1.8–2.5 µm in *H. rheicolor.* Tomentum is well developed in *H. attenuata,* thin and formed of less differentiated hyphal tufts in *H. rheicolor.* We have studied specimens of both species collected in Argentina, Brazil, China (Yunnan), Dominica, Guatemala, Guyana, India, Mexico,

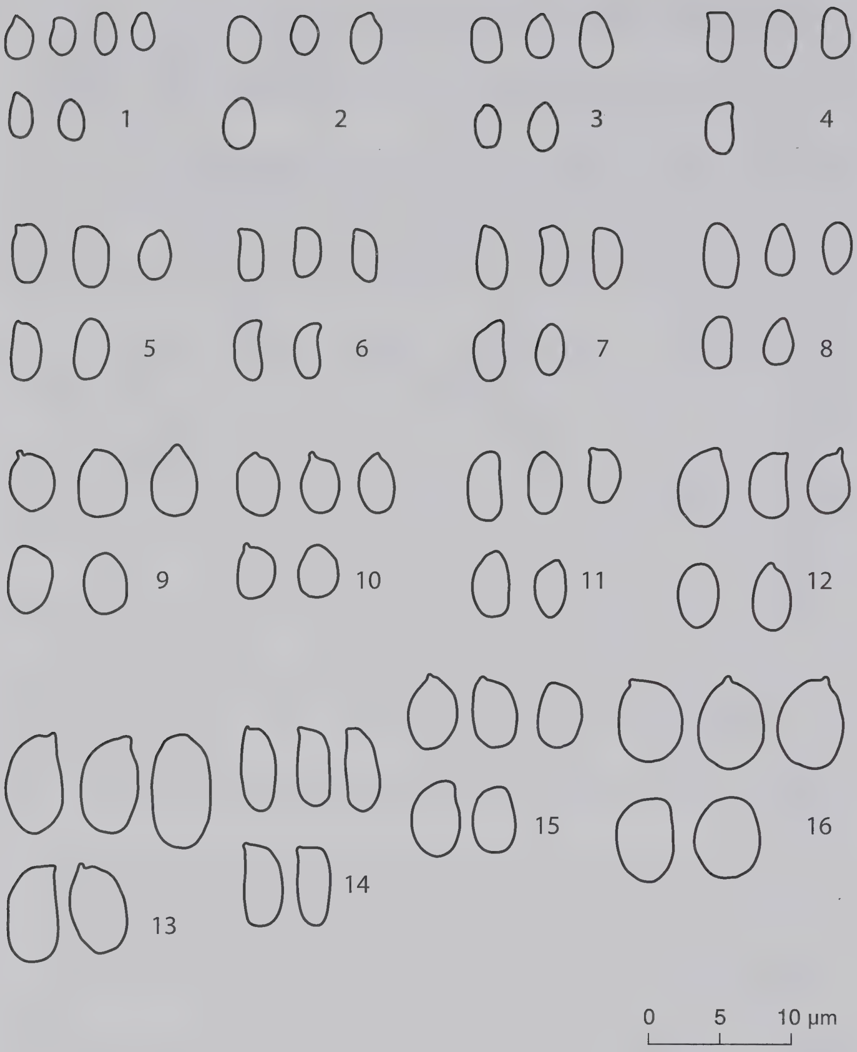


Fig. 1. Spores: *1 – Hymenochaete villosa, 2 – H. berteroi, 3 – H. subdissimilis, 4 – H. semistupposa, 5 – H. minuscula, 6 – H. attenuata, 7 – H. tomentelloidea, 8 – H. separabilis, 9 – H. geniculata, 10 – H. dissimilis, 11 – H. lignosa, 12 – H. flexosetosa, 13 – H. nothofagicola, 14 – H. legeri, 15 – H. muroiana, 16 – H. cervina*

Puerto Rico and Thailand, and found the variability of setal size to be much broader and sometimes overlapping in these species. Spore size is similar in the two species. We have seen only two specimens of "typical" *H. rheicolor* with spores bigger than in *H. attenuata* (see table below), and this difference is statistically insignificant when compared with spores of *H. attenuata*. However, most specimens of both species in various herbaria are sterile, and in the fertile specimens spores are usually partly damaged.

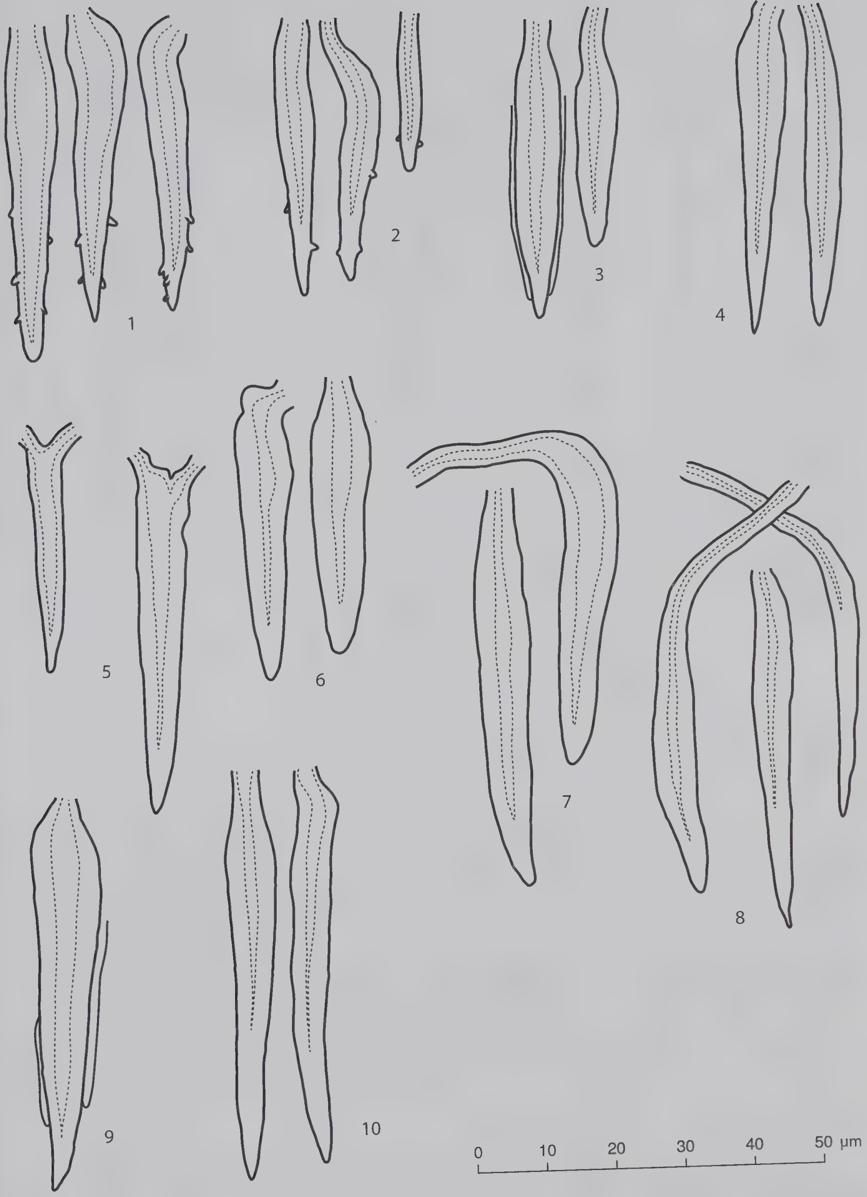

1
2
3
4
5
6
7
8
9
10
0
10
20
30
40
50 µm

Fig. 2. Setae: *1 – Hymenochaete tomentelloidea, 2 – H. separabilis, 3 – H. villosa, 4 – H. lignosa, 5 – H. muroiana, 6 – H. subdissimilis, 7 – H. flexosetosa, 8 – H. geniculata, 9 – H. attenuata, 10 – H. minuscula*

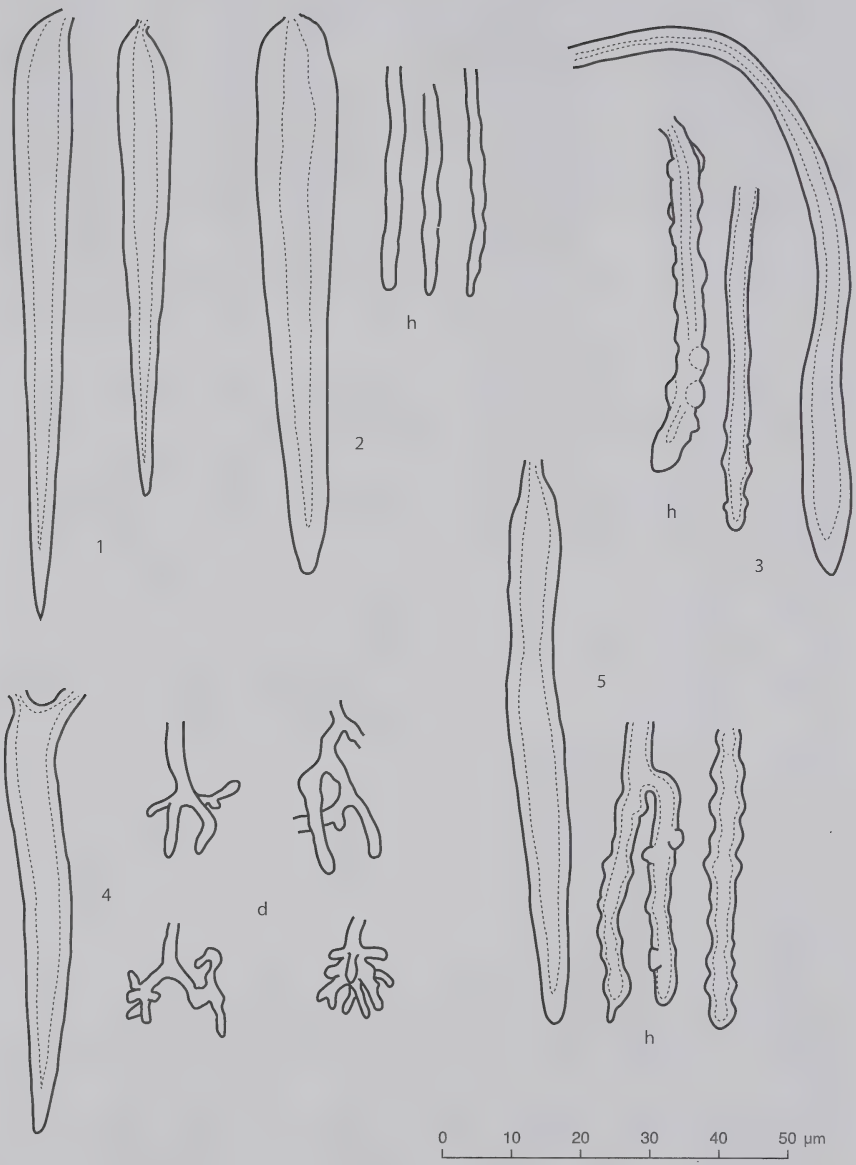

h
2
1
h
3
5
4
d
h
0
10
20
30
40
50 µm

Fig. 3. Setae and hyphidia: *1 – Hymenochaete dissimilis, 2 – H. innexa (h* – hyphidia), *3 – H. berteroi (h* – monilioid hyphidia), *4 – H. floridea (d* – dendrohyphidia), *5 – H. semistupposa (h* – monilioid hyphidia)

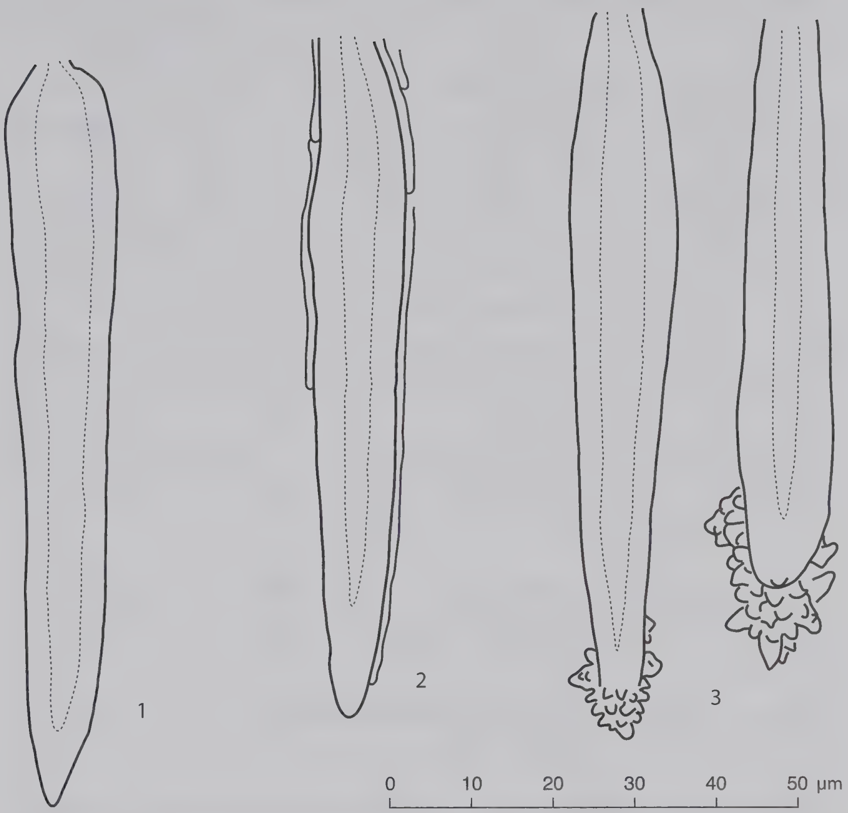


Fig. 4. Setae: *1 – Hymenochaete cervina, 2 – H. nothofagicola, 3 – H. legeri*

One of the additional characters of *H. attenuata* is differentiation of a hyphal layer near the hymenium. Hyphae of this layer are densely compacted in parallel, whereas in *H. rheicolor* they are interwoven.

It is possible to distinguish "typical" specimens of *H. attenuata* and *H. rheicolor,* but there are several "untypical" specimens with overlapping characters in herbaria, and the independence of these two species is doubtful.

Mean spore size and mean *Q* value in Hawaiian specimens of *H. attenuata* (*Q* value is the ratio of mean spore length to mean spore width):

3.72 x 1.90	1.95	RLG 21082
4.05 x 1.79	2.26	RLG 22743
4.09 x 1.95	2.10	RLG 21067
4.47 x 2.01	2.22	RLG 19253
4.48 x 1.88	2.38	RLG 17007
4.69 x 1.93	2.43	RLG 21081
4.96 x 1.90	2.61	RLG 18112

For comparison, the mean spore size and mean *Q* value in other specimens studied:

H. rheicolor	4.27 x 1.94	2.20	Mexico, BPI 278538
H. attenuata	4.34 x 1.85	2.35	Chile, BPI 330072
H. attenuata	4.34 x 1.86	2.35	Chile, BPI 33758
H. attenuata	4.54 x 2.00	2.27	India, TAA 103213
H. rheicolor	4.75 x 2.09	2.27	Guyana, BPI Lloyd 6734
H. rheicolor	4.80 x 1.93	2.49	Mexico, TN 18397
H. rheicolor	4.85 x 1.94	2.50	Mexico, XAL 1588
H. rheicolor	4.87 x 2.13	2.29	Mexico, TN 18369
H. attenuata	5.05 x 2.01	2.51	Thailand, O 18025
H. rheicolor	5.17 x 2.10	2.46	Dominica, BPI 297738
H. rheicolor	5.33 x 1.99	2.68	Puerto Rico, BPI 278545

H. attenuata sensu G. Cunn. found in New Zealand on dead twigs of *Nothofagus* spp. is a different species characterized with setae (55–)65–100 x (6–) 7–11(–14) µm, broadly ellipsoid or subcylindric spores with one side flattened, (5.5–) 6.0–8.0(–8.5) x (3–)3.5–4.5(–5.0) µm. Sparse dark brown thick-walled hyphidia 2.5–3 µm in diam are present in the hymenium. The New Zealand species will be described as a new one *(H. nothofagicola)* in the Appendix of this paper. Description of *H. attenuata* by Job (1991: 7–8) combines the data on *H. attenuata, H. attenuata* sensu G. Cunn. and a species collected in Switzerland and described by Job and Keller (1988).

2. **HYMENOCHAETE BERTEROI** Pat., Bull. Soc. Mycol. France 10: 78. 1894.

Description: see Parmasto, 2005. **Figs. 1,** ***2;*** **3,** ***3***

O'AHU. C.L SHEAR 113 (BPI 1100595), on *Acacia koa*, Pupukea Forest Reserve, 31 Jan (1928?).

Comments. Closely related to *H. semistupposa,* which differs by absence of setal hyphae and having spores 3.2–4.5 x 1.7–2.2 µm.

3. **HYMENOCHAETE CERVINA** Berk. & M.A. Curtis, J. Linn. Soc., Bot. 10: 334. 1868.

Figs. 1, ***16;*** **4,** ***1***

Basidiomata perennial, effused, adnate, woody hard, up to 500–1100 µm thick, as rounded patches up to 5 cm or more in length. Hymenium even, umber (M: 5–7.5 YR 4/6 to 5/4; K & W: 7 E 4 – 7 F 5); margin abrupt.

Cortex present but not easily distinguishable, usually observable as a dark line near the substrate; hyphal layer absent; setal layer very thick; dark line above the hymenium absent.

Hyphal system subdimitic; (pseudo)skeletal hyphae ascending or vertically interwoven, densely agglutinated, with thick walls, brown, sparsely branched, 3–5 µm in diam; generative hyphae innumerous, thin-walled, branched, subhyaline, 3–5 µm in diam; setae numerous, narrowly conical or spine-like, at base arising from a dense node of thick-walled hyphae, straight, 70–100(–120) x 7.5–12(–14) µm, emerging up to 40 µm above the hymenium, with a hyphal sheath which is finely encrusted in old setae; cystidia absent; hyphidia scattered, cylindrical, with brown thickened walls, in upper part sometimes slightly sinuous, 2.5–3.5 µm in diam; basidioles present, thin-walled; basidia clavate or slightly subutriform, 20–25(–30) x 7–8.5 µm, with 4 thin sterigmata about 4 µm long; spores broadly ellipsoid, with one side slightly flattened, mostly with a large guttule, (5.7–)6–7 x (4.0–)4.3–5(–5.2) µm.

Causes a fibrous white rot of wood.

KAUA'I. RLG 20644 on *Cryptomeria japonica,* Lehua puhi Trail, Koke'e State Park.

Comments. This Hawaiian specimen is typical for *H. cervina.* Presence of crystalline matter in the setal layer indicated for this species by Léger (1998: 86) is a variable character in *H. cervina.* Spores have rarely been observed in specimens kept in world herbaria but the Hawaiian collection has numerous spores. Their mean size is 6.39 x 4.60 µm and the length/width quotient is 1.39 (average of 22 spores measured). *H. cervina* has been found in Australasia (New Zealand), South America, Caribbean and North America. Data on occurrence in East Tropical Africa (Uganda) are doubtful.

4. HYMENOCHAETE DISSIMILIS G. Cunn., Trans. Roy. Soc. New Zeal. 85 (1): 44. 1957.

Figs. 1, *10*; 3, *1*

Basidiomata perennial, effused, closely adnate, hard when dry, up to 1500 µm thick; hymenium smooth or tuberculous, deeply cracked, when young grayish Cinnamon (M: 5.5 YR 5/6; K & W: 6 D 4), then dark Vinaceous Buff (M: 7.5 YR 5.5/4; K & W: dark 6 D 3); margin indistinct, then abrupt.

Tomentum and cortex absent, hyphal layer thin and soon disappearing; context composed of thickening, indistinctly stratose setal layer.

Hyphal system monomitic; hyphae densely agglutinated with brown resinous matter, 2.5–4.5 µm in diam, brownish to brown, thick-walled, septate, branched; in context and hymenium crystalline matter usually present; setae numerous, of variable size, (35–)45–80 x 5–9 µm, projecting to 50 µm above hymenium, subulate, with acute tip, straight, usually with a thin hyphal sheath, without incrustation; cystidia and hyphidia absent; basidioles with thin walls at base slightly thickened and yellowish, 18–25 x 3.5–4.5 µm; basidia subutriform, (15–)18–22(–25) x 4–5 µm, with 4 sterigmata about 4 µm long; spores ellipsoid, with one side flattened, 4.2–5.4 x 2.5–3.2 µm.

HAWAI'I. RLG 17624, 17741 on *Acacia koa,* Keanakolu Rd.

Comments. This species has been described from New Zealand and found later only in Réunion. All three specimens collected on Hawai'i differ from the previously published descriptions by lack of crystalline masses in the setal layer. We do not consider this character to be a taxonomically important one.

H. dissimilis is externally very similar to *H. unicolor.* Both may have very thick cracked basidiomata. The last named species differs in having smaller setae (30–)35–50 x 5–7 µm and spores 3.4–4.8 x 2.2–2.8 µm.

Mean spore size and mean Q value of *H. dissimilis:*

4.66 x 2.81	1.66	RLG 17624
5.03 x 2.90	1.74	RLG 17741

5. HYMENOCHAETE FLEXOSETOSA Parmasto, sp. nova

Figs. 1, *12;* 2, *7*

Basidiomata annua, pileata, flabelliformes, imbricata, ad 1 cm longa et 450 µm crassa. Tomentum et stratum hypharum adsunt, cortex et textus setarum desunt. Systema hypharum monomiticum; hyphae tunicis incrassatis, ramosae; setae subulatae, 40–70 *x 7–12 µm, subacutae, sine incrustatione, setae nonnullae in parte basale ex hyphis setoideis breves 20–40(–60) x 3–5 µm adscedentia; cystidia et hyphidia desunt; basidioli tunicis incrassatis basim tenuiter granulosis; basidia 20–25 x 4–5 µm; basidiosporae late ellipsoideae, (4.8–)5–5.8(–6.2) x 3–3.5(–3.7) µm. A* H. attenuata *et* H. rheicolore *hyphis setoideis praesentis atque sporis latis differt.*

Holotypus: Hawai'i, on *Psidium guajava,* R.L. Gilbertson 18932, Honokaia Boy Scout Camp (ARIZ; isotypus: TAA).

Etymology: *flexuosus*, flexuous; *seta*, seta.

Basidiomata annual, pileate, soft, papery-coriaceous and flexible when dry; pilei flabelliform, 0.3–1 cm long, 200–450 µm thick, imbricate, sometimes seemingly effused-reflexed but then umbonate (attached in one point); pileal surface velutinous or tomentose, concentrically sulcate and zonate, coarsely radiately fibrillate (silky-fibrous), dull Fulvous Umber (M: 7.5 YR 4/6; K & W: 6 D 5); margin thin, lobate, concolorous with the pileal surface or lighter coloured; hymenium smooth, not cracked, grayish Fulvous (M: 7.5 YR 5/6; K & W: 6 D 4).

Tomentum present as loosely interwoven hyphae, 80–200 µm thick; cortex absent; hyphal layer composed of radiately interwoven generative hyphae and few short setal hyphae bending to setae; setal layer absent.

Hyphal system monomitic; hyphae with thickened walls, brownish (in tomentum thick-walled and brown), sparsely branched, with septa, in hyphal layer 2.5–3.5 µm, in tomentum 3.5–5 µm in diam; setae innumerous or numerous, subulate, straight, bluntly acute, 40–70 x 7–12 µm, emerging 10–25 µm above the hymenium, with a thin hyphal sheath, without incrustation; some setae are L-shaped, originating in the context from a short setal hypha like horizontal part 20–40(–60) µm long and 3–5 µm in diam; this continues as a usual thick-walled generative hypha; basidioles with thickened and brownish base with rough (finely encrusted) walls, 25–30 x 3.5–4 µm; basidia 20–25 x 4–5 µm, with 4 thin sterigmata; spores broadly ellipsoid, with one side flattened, (4.8–)5–5.8(–6.2) x 3–3.5(–3.7) µm.

Causes white fibrous rot of wood.

HAWAI'I. RLG 18932 on *Psidium guajava,* Honokaia Boy Scout Camp.

Comments. This new species is externally very similar to *H. attenuata* and *H. rheicolor* which differ in spore form and width (1.7–2.3 µm) and absence of setal hypha-like base of L-shaped setae characteristic for *H. flexosetosa.* Mean size of spores of the type of *H. flexosetosa* is 5.40 x 3.21 µm; *Q* = 1.68.

An almost identical specimen has been collected 18 Sep 1956 by S. Ahmad from Pakistan on branches of *Viburnum* sp. and identified by him as *H. rheicolor* (LY 2284). It is sterile, with no spores.

6. HYMENOCHAETE FLORIDEA Berk. & Broome, J. Linn. Soc., Bot. 14: 68. 1873.

Fig. 3, *4*

Basidiomata effused, closely adnate, 100–200 µm thick (up to 500 µm according to Léger, 1998: 140); hymenium smooth, usually not cracked, bright Sienna (or reddish brown) (M: 5 YR 5-6/8; K & W: 7 (C–D) 7; specimens described by Léger M: 5 YR 4/3–4); margin very narrow.

Tomentum and cortex absent; hyphal layer present, of densely interwoven hyphae.

Hyphal system monomitic; hyphae interwoven, distinct, 2.5–4.5 µm in diam, brown, with thick walls, septate, branched; setae numerous, (55–)60–85(–100) x 6–9(–10) µm, broadly subulate, with bluntly acute tip, straight, usually with a thin hyphal sheath, some encrusted with granules, projecting to 40 µm above the hymenium, at base usually surrounded with arbuscular hyphidia; cystidia absent; hyphidia very numerous, with brown thick-walled stem 3–4 µm in diam, in subhymenium and hymenium dendroid, repeatedly branched, in setal layer very numerous head-like brown arbusculi 10–25 µm in diam with very numerous short side branches about 1 µm in diam; basidia not seen but according to Léger (1998: 140) 15 x 4 µ; spores cylindrical or ellipsoid, 6–7 x 2.5–2.8 µm.

HAWAI'I. RLG 19000: on *Metrosideros polymorpha,* Thurston Lava Tube, Hawai'i Volcanoes Nat. Park (HVNP).

Comments. This is the only *Hymenochaete* species with heavily branched dendrohyphidia in Hawai'i. Somewhat similar is *H. sphaericola* Lloyd *(H. cruenta* (Pers.: Fr.) Donk *p.p.*), a species widely distributed in East and South-East Asia and Australasia that has dendrohyphidia with innumerous long (5–25 µm) branches never forming arbusculoid heads. *H. floridea* has Asia Tropical–North-Central Pacific distribution.

7. HYMENOCHAETE GENICULATA Parmasto, **sp. nova** **Figs. 1,** *9;* **2,** *8*

Basidiomata perennia, effusa, coriacea, 100–250, deinde ad 500 µm crassa. Hymenium laeve vel leviter colliculosum, atro-ochraceum, vetustus crevisum. Tomentum et cortex adsunt; stratum hypharum crassum, ad stratum setarum indistinctum transiens, parte basale cum setis adscedentis atque hyphae setales vel setae hyphales nonnullis ad 80 x 6 µm. Systema hypharum subdimiticum; hyphae tunicis incrassatis brunneis, leviter ramosae non densiter intermixtae; setae numerosae, anguste fusoideae, 40–70 x 7–12 µm, partim breve stipitatae, aliae basim geniculate arcuatae, acutae, sine incrustatione; basidioli numerosi, subhyalini; basidia cylindraceo-clavati, 20–30 x 4–5 µm; basidiosporae breve cylindraceae vel subellipsoideae, 4.5–6 x 2.3–3(–3.3) µm. Species similis H. stratura stratis hypharum hyphis paralleliter densiter contextis atque sporis minutis 3.5–4 x 2–2.5 µm differt.

Holotypus: Kaua'i, Koke'e St. Park, on *Metrosideros polymorpha,* 5 Jan 2000, R.L. Gilbertson 22797 (ARIZ; isotypus: TAA).

Etymology: *geniculatus,* (setae) bent like a bent knee.

Basidiomata perennial, effused, adnate, when old detachable as small pieces, coriaceous, 100–250, later up to 500 µm thick, as rounded patches, then confluent and up to 10 cm long. Hymenium even or slightly tuberculose, yellowish brown, bronze brown or dark ochraceous with an isabelline tint (M: 10 YR 5/8, 10 YR 5/6, when old umber – 7.5 YR 4/4); K & W: 5 (D–E) 5, when old 6 F 6), later with a few deep crevices; margin indistinct and more lightly coloured, then abrupt.

Tomentum and cortex absent; hyphal layer thick, in lower part with scattered setae (with gradual transition to the indistinct setal layer), with a few horizontal setae or short setal hyphae, in old specimens more dense and may be called setal layer.

Hyphal system subdimitic; hyphae in the hyphal layer almost loosely interwoven, with thickened brownish walls (some hyphae thin-walled and subhyaline), sparsely branched, with septa, 2.5–3.5(–4) µm in diam; setae numerous, narrowly fusoid, (35–)40–60 x 4–7(–8) µm, partly with a slightly sinuous thick-walled stipe, others angled (geniculate) near the base, with an acute tip, naked or covered with a thin hyphal sheath, projecting up to 20 µm above the hymenium, setae embedded near to the substrate ascending and up to 75 µm long; setal hyphae (embedded horizontal setae) 40–80 µm long, 3.5–6 µm in diam; basidioles numerous, subhyaline, 20–30 x 3.5–4.5 µm; basidia cylindrical-clavate, 20–30 x 4–5 µm, with 4 sterigmata 2.5–3.5 µm long; spores short-cylindrical or almost ellipsoid, some with one side concave, 4.5–6 x 2.3–3(–3.3) µm.

Causes fibrous white rot of wood.

HAWAI'I. RLG 20478, on *Coprosma montana,* Kipuka Puaulu, HVNP; RLG 17378, 22365 on *Sapindus saponaria,* Kipuka Ki, HVNP. KAUA'I. RLG. 22797 (holotype), on *Metrosideros polymorpha,* Lehua Puhi Trail, Koke'e State Park.

Comments. The species is possibly closely related to the *H. stratura* G. Cunn., found twice in New Zealand on wood of *Podocarpus* species; *H. stratura* differs in having a hyphal layer composed of densely parallel hyphae and small spores 3.5–4 x 2–2.5 µm.

Mean spore size and mean Q value of *H. geniculata:*

4.98 x 2.81	1.77	RLG 22797
5.30 x 2.75	1.93	RLG 17378
5.55 x 2.55	2.17	RLG 22365

8. HYMENOCHAETE INNEXA G. Cunn., Trans. Roy. Soc. New Zeal. 85 (1): 47. 1957.

Fig. 3, *2*

Basidiomata perennial, effused, closely adnate, hard when dry, 50–100 µm thick; hymenium smooth, not cracked, light Umber (M: 5 YR 5/4; K & W: 6 D 4; New Zealand specimens described by Léger (1998: 165) as reddish brown, M: 5 YR 4/4); margin very narrow, thin, arachnoid-fibrillose, Sienna (M: 7.5 YR 6/6), then indistinct.

Tomentum, cortex and hyphal layer absent.

Hyphal system monomitic; hyphae interwoven, distinct but partly agglutinated with brown resinous matter, 2–3 µm in diam, brownish, with thickened walls, septate, branched; setae numerous, 50–85 x (7–)8–13(–14) µm, broadly subulate, with bluntly acute tip, straight, usually with a thin hyphal sheath, some encrusted with granules, projecting to 40 µm above the hymenium; cystidia absent; hyphidia with brown thickened walls (but when new basidia are developing, then hyaline or absent), some slightly sinuous, 2–3 µm in diam; basidia subutriform, with slightly thickened walls at base, 15–20 x 4–5 µm, with 4 thin sterigmata; spores cylindrical, straight or slightly curved, 5.5–7(–7.5) x 1.8–2.5 µm.

Causes white pocket rot of wood.

O'AHU. RLG 18411 on *Eucalyptus* sp., Manoa Falls Trail.

Comments. There is only one other *Hymenochaete* species with non-monilioid brown hyphidia in Hawai'i, *H. cervina,* but it has broadly ellipsoid spores (5.7–)6–7 x (4.0-)4.3–5(–5.2) µm. *H. innexa* has been previously found in China, Japan, India, Australia and New Zealand; isotypes in BPI studied by E. P. The Hawaiian specimen has only few spores; mean size of 11 spores measured is 6.67 x 2.16 µm; *Q* = 3.1.

9. HYMENOCHAETE LEGERI Parmasto, sp. nova Figs. 1, *14;* 4, *3*

> *A* H. corrugata *(Fr.: Fr.) Lév. setis longis robustis (60–)75–100(–110) x 10–15 μm apicis crystallis multis tectis, sporis grandis cylindricaceis vel suballantoideis 5.5–8.2 x 2.2–3.2 μm nec non hymenio pallide griseo vel albido differt.*
>
> **Holotypus**: Hawaiian Islands, Moloka'i Is., Kamakou Rd., on *Eucalyptus* sp., 9 Jan 2000 R.L. Gilbertson 23032 (ARIZ; isotype in TAA).
>
> **Etymology**: Jean-Claude Léger (2 Oct 1938 – 16 July 1999), distinguished French mycologist, author of a fundamental monograph of world *Hymenochaete.*

Basidiomata annual, effused, closely adnate, crustose, woody hard, 100–500 μm thick (when old up to 1 mm and sometimes indistinctly stratified), as rounded patches 0.5–2 cm long, then confluent and up to 10 cm long; hymenium even or somewhat tuberculose when old, later with some irregular deep fissures, pale Mouse Gray, Vinaceous Buff, pale Vinaceous Buff or whitish (M: 5–10 YR 7–8/2 to 8/1–3; K & W 5 B 2 or (5–6) (B–C) 2), when old and sterile dark Fulvous to Umber or Sepia (M: 5 YR 4–5/4–6; K & W 6 D 6); margin clearly delimited, when young narrowly white and fimbriate, soon abrupt, usually with a narrow brown(ish) strip (M: 5–7.5 YR 4/6; K & W: 6 D 6 – 6 E 7).

Tomentum absent; cortex absent or present and then 10–20 μm thick; hyphal layer 30–80 μm thick, sometimes almost absent; setal layer thickening; dark line above the hymenium absent.

Hyphal system subdimitic; hyphae in hyphal layer at first not densely, then densely interwoven; generative hyphae with thin or slightly thickened walls, subhyaline, sparsely branched and septate, 2.5–4 μm in diam; skeletoids sparsely branched, with thickened walls, brownish, 2.5–4 μm in diam; setae numerous or crowded, hymenium composed of basidioles, basidia and innumerous thin- or thick-walled hyphidia; setae broadly conical, at base arising from a dense coralloid node of brown thick-walled hyphae, straight, (60–)75–100(–120) x 10–15 μm, projecting 30–60 μm above the hymenium, covered with a hyphal sheath, acute or almost blunt tip usually covered with a conical conglomerate of crystals; hyphidia rare, cylindrical, brownish, with thickened walls, 2–3 μm in diam; basidioles thin-walled; basidia subutriform, 20–25 x 4.5–6 μm, with thin hyaline walls, with (2) 4 sterigmata 3–4 μm long; spores cylindrical or suballantoid, 6–8 x 2.2–3.2 μm.

Causes white pocket rot of wood.

> HAWAI'I. RLG 17184 on hardwood, RLG 17193 on *Grevillea robusta,* RLG 17194 on *Psychotria hawaiiensis,* RLG 18651 on *Psidium guajava*, Kalopa State Park; RLG 17553 on *Casuarina* sp., RLG 17806, 17858, 18202 on *Psidium guajava,* RLG 17859, 18242, 22239 on *Eucalyptus* sp., RLG 18924 on *Toona ciliata;* Honokaia Boy Scout Camp; RLG 20977, 20989 on *Toona ciliata,* Stainback Highway; RLG 17246 on *Mangifera indica,* Rainbow Falls; RLG 19428 on *Mangifera indica,* Kapoho Rd.; RLG 20727 on *Acacia koa,* Keanakolu Rd.; RLG 23308 on *Coprosma montana*, Kipuka Puaulu, HVNP; RLG 16826 on *Sapindus saponaria*, Kipuka Puaulu, HVNP; RLG 22250 on *Schinus terebinthifolia,* RLG 22254 on *Cerecropia obtusifolia*, Manuka State Park. KAUA'I. RLG 21072 on *Acacia koa,* Makaha Rd.; RLG 21198 on *Metrosideros polymorpha*, Lehua Puhi Trail, Koke'e State Park; RLG 17945, 21260, 22726 on *Pinus elliottii*, Makaha Rd.; RLG 20617, 21055 on *Sequoia sempervirens;* Koke'e State Park; RLG 21044 on *Eucalyptus* sp., Kukiolona Park, Kalaheo; RLG 21168: on *Metrosideros polymorpha,* Ohelo Berry Flat Trail, Koke'e State Park; RLG 21226 on *Acacia koa,* Makaha Rd.; RLG 22727 on hardwood, Makaha Rd.;

RLG 22787 on *Nestegis sandwicensis*, RLG 22803 on *Metrosideros polymorpha*, Lehua Puhi Trail, Koke'e State Park; RLG 22845 on *Acacia melanoxylon*, Ohelo Berry Flat Trail, Koke'e State Park; RLG 21251 on *Pinus elliottii*, Miloli'i Rd. **Maui.** J.E. Adaskaveg 1469 on *Spathodea campanulata*, Mile 4, Hana Highway; RLG 19172 on *Psidium guajava*, Maluhia Boy Scout Camp; RLG 21475, on *Pinus pinaster*, Waihou Spring Trail, Olinda; RLG 21524 on *Aleurites molluccana*, Makemakeole Trail, West Maui; RLG 23150, 23158 on *Araucaria heterophylla*, Maluhia Boy Scout Camp. **Moloka'i.** RLG 21449 on *Casuarina* sp., Pala'au State Park; RLG 21337, 23032, 22965, 22978, 23044, 23048, 23053 on *Eucalyptus* sp., Kamakou Rd.; RLG 19204 on hardwood, Kamakou Rd.; RLG 19353 on *Acacia mearnsii*, Kamakou Rd.; RLG 23002 on *Araucaria heterophylla*, Kamakou Rd.; RLG 21411 on *Pinus pinaster*, Kamakou Rd,; RLG 22947 on *Casuarina* sp., Kalaupapa Overlook. **O'ahu.** C.L Shear, BPI 11006101, on *Hibiscus tiliaceus*, Tantalus Rd.; Don Hemmes 65 on *Syzygium cumini*, Pali; RLG 16931 on hardwood, Manoa Falls Trail; RLG 18331, on *Schinus terebinthifolia*, Pu'u Ohia Trail; RLG 18346: on *Psidium guajava*, Manoa Cliffs Trail.

Found also in India (Tamil Nadu St., Kalakad Sanctuary, 13 Feb 1979 E. Parmasto, TAA 103250), Taiwan (Wu 880501/26, TAA) and Azores (on *Cryptomeria*, B. Spooner, K).

Comments. The new species is well characterized by broad setae with a tip usually heavily covered with crystals, relatively big suballantoid spores and (when not very old or dead) alabaster or marble white hymenium. It seems to be closely related to the widespread (but not reported in Hawai'i) species *H. corrugata* that also has relatively big setae with nodose hyphal base and granulose tip, but has a much darker pinkish gray hymenium. That species has setae 35–80(–100) µm long, spores of similar form but smaller (4.5–6.5 x 1.5–2.3 µm). Several species of *Hymenochaete* have whitish gray hymenium covered with numerous crystals when basidiomata are old, sterile or half-dead. In *H. legeri,* pale colour of hymenium caused by abundant crystals is a sign of intensive growth and in many cases also of presence of ripe basidia and spores.

In old specimens of *H. legeri* the hyphal layer is almost indistinguishable from the setal layer, and cortex is hardly observable. In sterile and no longer growing specimens the hymenium turns brown and the characteristic pale (whitish) colour fades off.

H. legeri has Macaronesia–Asia Tropical–North-Central Pacific distribution.

Mean spore size and mean *Q* value of *H. legeri:*

6.30 x 2.71	2.32	RLG 18346
6.31 x 2.64	2.39	RLG 18331
6.31 x 2.48	2.54	RLG 19172
6.34 x 2.41	2.63	RLG 23032
6.48 x 2.56	2.53	RLG 17194
6.56 x 2.36	2.78	RLG 18924
6.68 x 2.58	2.60	RLG 22978
6.77 x 2.68	2.53	Azores (K)
6.92 x 2.57	2.69	RLG 21475
6.94 x 2.49	2.78	RLG 18924
7.03 x 2.64	2.67	BPI, Shear 1100610
7.05 x 2.61	2.70	RLG 23048
7.17 x 2.63	2.73	RLG 23044
7.18 x 2.57	2.79	RLG 22965
7.18 x 2.63	2.73	RLG 21055

7.22 x 2.63	2.75	RLG 22947
7.25 x 2.53	2.87	RLG 21449
7.37 x 2.68	2.75	RLG 21044
7.37 x 2.87	2.56	Adaskaveg 1469
7.92 x 2.67	2.97	RLG 17184

10. HYMENOCHAETE MINUSCULA G. Cunn., Trans. Roy. Soc. New Zeal. 85 (1): 48. 1957.

Figs. 1, *5;* **2,** *10*

Basidiomata perennial, effused, adnate, woody hard, 150–450 µm thick (50–150 µm in specimens found in New Zealand, Africa and America), when old with innumerous deep cracks. Hymenium smooth, dark grayish Ochreous (M: 10 YR 5/6; K & W: 6 D 4); margin abrupt.

Cortex, tomentum and hyphal layer absent; setal layer indistinctly stratose, with scattered crystals; dark line above the hymenium absent.

Hyphal system monomitic; hyphae distinct, densely interwoven, with thin or thickened brownish walls, sparsely branched, septate, 2.5–4 µm in diam; setae numerous, subulate, acute, straight or some slightly curved and undulate, 35–60(–65) x 4–7 µm, usually with a thin hyphal sheath, emerging up to 40 µm above the hymenium; cystidia and hyphidia absent; basidioles 18–25 x 3–3.5 µm, with thickened base walls encrusted with granules; basidia subutriform, 15–22 x 3.5–4 µm, with 4 thin sterigmata; spores ellipsoid or short-cylindrical, with one side flattened or slightly concave, 4.2–5.2 x 2.2–2.5 µm.

Causes a fibrous white rot of wood.

HAWAI'I. RLG 17722: on *Acacia koa*, Keanakolu Rd..

Comments. Ochraceous or yellowish brown colour characterizes this species. It has been found also in New Zealand, Western Indian Ocean region of Africa, and in South America, i.e, has a Gondwanan distribution type.

Mean spore size and mean *Q* value of *H. minuscula:*

4.67 x 2.37	1.98	RLG 17722

11. HYMENOCHAETE MUROIANA I. Hino & Katum. in Hino, Icones Fung. Bambus. Japan 237. 1961.

Figs. 1, *15;* **2,** *5*

Basidiomata annual, effused, adnate, when older detachable as small fragile pieces, coriaceous, hard when dry, 30–50(–100 ?) µm thick, as rounded patches 2–5 cm long, later sometimes confluent and up to 15 cm long. Hymenium even, vinaceous buff, fawn or dark fawn, when old dark fawn or grayish umber (M: 5 YR 5/4 or 6/1 – 7/2, when old and sterile 5 YR 4.5/2; K & W: 7 C 2 – D 3, when old 7 E 4–5); margin thin, indistinct, then distinctly delimited, concolorous with the hymenium.

Tomentum, cortex and hyphal layer absent; setal layer very thin, 10–20 µm thick; dark line above the hymenium absent.

Hyphal system monomitic; hyphae in setal layer densely interwoven, agglutinated, with thickened or thick walls, brownish, with rare septa and branches, (2–)2.5–4 µm in diam; hyphal layer composed of loosely interwoven, richly branched and septate brownish hyphae with thickened or thick walls, 2.5–4.5 µm in diam; setae numerous; hymenium composed of basidioles and basidia; cystidia and hyphidia absent; setae narrowly (sometimes broadly) subulate, usually partly bifurcate at base, with slightly curved upper part and acute or almost blunt tip, (30–)40–50(–60) x (5–)6–8(–9) µm, naked (without a hyphal sheath), projecting up to 20(–30) µm above the hymenium; basidioles thin-walled; basidia subutriform, 15–20 x 4.5–6 µm, with thin hyaline walls, with 4 sterigmata 3–4 µm long; spores broadly ellipsoid, 4–6 x (2.5–)3–3.5 µm.

Causes fibrous white rot of wood.

> **Hawai'i.** RLG 21011, 21019, 21028 on *Bambusa vulgaris*, Waipio Ridge Trail; RLG 21013, 21017 on *Phyllostachys nigra*, Waipio Ridge Trail. **Maui.** C.L. Shear and N.E. Stevens, BPI 1199597; West Maui, Pogues Ditch, 2 Jan 1928, no other data (BPI 1100597).

Comments. This species has been described from Japan, and found later only in Taiwan (NMNS, S.H. Wu 880501-26, unpublished data), Indo-China and Malesia (Parmasto, 2005). Most collections are from dead (fallen) stems of bamboo.

In the original description of *H. muroiana* and its synonym *H. iriomotensis* I. Hino & Katum. (Hino & Katumoto, 1964) as well as in the redescription of the types by Léger (1998: 199), the spores are described as (3–)3.5–4(–4.5) x (1.8–)2–2.6 µm and setae as very numerous and smaller than in most of our specimens (Léger: (16–)18–35 x (4.5–)5–6(–6.5) µm). However, some old sterile specimens collected from Taiwan and Hawai'i (BPI 1100597) studied by us have densely crowded, short conical setae 30–40 x 7–12 µm; setal variation seems to be related to the state of development of the basidiomata. The types of this species were described by Léger (1998: 198, 200) as soot brown *(brun suite,* 7.5 YR 5/2); as in most species of *Hymenochaete,* the colour depends on presence or absence of basidia and basidioles. In the Hawaiian collections, both sterile umber fawn and fertile vinaceous buff specimens are represented.

One of the Hawaiian specimens (RLG 21011) differs from the others by thickness (up to 250 µm), dark brown colour (7.5 YR 4/6), setae 35–50(–55) x 5–7(–8) µm, small spores 3–4 x 1.9–2.5 µm (mean size: 3.47 x 2.15 µm, $Q = 1.62$) and substrate *(Coprosma montana* of the family *Rubiaceae).* According to the spore size, this collection is close to the Japanese and Sulawesi specimens of *H. muroiana.* It may be possible that there is more than one species in this group.

Mean spore size and mean *Q* value of *H. muroiana:*

5.36 x 3.50	1.54	RLG 21028
5.70 x 3.65	1.39	RLG 21019

12. HYMENOCHAETE SEMISTUPPOSA Petch, Ann. Roy. Bot. Gard., Peradeniya 9: 278. 1925.

Figs. 1, *4;* **3,** *5*

Basidiomata annual (but sometimes layered), effused, adnate but easily detachable, soft membraneous, 200–500 µm thick; first 3–10 mm in diam, with pennately fimbriate margin, then confluent. Hymenium even (under looking glass pubescent), in old specimens sometimes minutely cracked, sepia to dark brown (M: 2.5–5 YR 4/3, K & W: 6 E 6); margin when young pennate-fimbriate with fibrils 1–2 mm long, not attached to the substrate, margin and very young basidiomata sienna or fulvous to sienna (M: 5 YR 5–6/8; K & W: Cinnamon or Raw Sienna, 6 D (6–7)).

Tomentum absent or present as abhymenial hairs; cortex present, 40–150 µm thick; context composed of cinnamon or fulvous hyphal layer 100–200 µm and thin to thick setal layer up to 200 µm thick; dark line above the hymenium absent.

Hyphal system monomitic; hyphae of the fibrillose margin parallel, with thickened walls, yellow, rarely septate, some slightly flexuous; cortex composed of densely agglutinated parallel brown, thick-walled hyphae 3.5–5 µm in diam; hyphal layer composed of loosely interwoven, richly branched, septate brown hyphae with thickened or thick walls, 2.5–4.5 µm in diam, many hyphae slightly monilioid and with punctually thickened walls; setal layer made of thickening hymenium, between setae are vertically situated, densely parallel, frequently septate, yellow or brownish, slightly monilioid hyphae; hymenium composed of setae, numerous hyphidia and basidioles, and few basidia; setae numerous, conical, straight, with acute tip, (40)45–70(–80) x (6–)7–9.5(–12) µm, mostly with a hyphal sheath, projecting up to 40 µm above the hymenium; hyphidia, basidioles and basidia similar to each other, 18–25 µm long and 2.5–4 µm (basidia up to 6 µm) in diam, with walls thickened and brownish at base, developing from hyphae with numerous septa; many hyphidia monilioid and/or with thickenings on walls; basidia cylindrical, with 4 sterigmata 3–4 µm long; spores ellipsoid or short cylindrical, one side slightly concave or flattened, 3.2–4.5 x 1.7–2.2 µm.

> HAWAIIAN IS. 1921 F.L. Stevens 871, 878 (BPI 277727, 277717, identified as *H. cinnamomea). HAWAI'I.* RLG 18934 on *Eucalyptus* sp., Honokaia Boy Scout Camp; RLG 17442 on *Metrosideros polymorpha*, Thurston Lava Tube, HVNP; RLG 16978, 16983 on *Metrosideros polymorpha*, Mile 21, Saddle Rd.; 18085, 20746, 20757, 20763 on *Metrosideros polymorpha,* Mile 18, Saddle Rd.; RLG 19047 on *Metrosideros polymorpha*, Manuka State Park. KAUA'I. RLG 20580 on *Acacia melanoxylon,* Ohelo Berry Flat Trail, Koke'e State Park; RLG 22828 on hardwood, Lehua Puhi Trail, Koke'e State Park. O'AHU. C.L. Shear (BPI 1100598) on *Metrosideros polymorpha*, Manoa Valley.

Comments. Cunningham (1957) and Job (1987) found that the basidiomata of this species may be layered. In several Hawaiian specimens new basidiomata are growing on old (dead?) ones, in the beginning as small isolated "islands". The "layers" (basewalls) of the basidiomata are not the result of uniform thickening of old ones.

H. semistupposa has been found in Southern Africa, East Tropical Africa (Kenya), Asia Tropical (India and Sri Lanka) and Australasia. Almost all known substrates belong to the tropical family *Myrtaceae*. These data suggest that this fungus belongs to the Gondwanan historical type of distribution.

Mean spore size and mean Q value of *H. semistupposa:*

3.56 x 1.83	1.95	RLG 16983
3.57 x 1.81	1.97	RLG 18934
3.75 x 1.93	1.94	RLG 20580
3.93 x 1.82	2.17	RLG 18085
4.13 x 1.93	2.14	RLG 22828

13. HYMENOCHAETE SEPARABILIS J.C. Léger, Bull. Soc. Mycol. France 97 (1): 7. 1981.

Figs. 1, *8;* **2,** *2*

Basidiomata annual or perennial, effused, adnate but separable as pieces when dry, soft or coriaceous, when young about 60 µm thick, then 200–400 µm, as rounded patches 0.5–5 cm in diam, later confluent and up to 10 cm long; hymenium even, in older parts irregularly cracked; light Umber (M: 5 YR 4.5–5/3–4; African specimens 10 YR 6/8, later 7.5 YR 5/6; K & W: 7 E 6); margin thin, slightly fibrillose, then abrupt, distinct, lighter coloured than hymenium; context lighter coloured than hymenium, dark Sienna (M: 5 YR 5/7; K & W: 7 D 6).

Tomentum and cortex absent; hyphal layer present but weakly differentiated from the thickening setal layer; dark line above the hymenium absent.

Hyphal system monomitic; hyphae brownish, with thickened walls, occasionally branching, septate, 2–4 µm in diam, in hyphal layer loosely, later densely interwoven; setae numerous, subulate, subfusoid, with acute tip, 25–45 x 5–7(–8) µm, straight or some slightly curved, not encrusted, usually with a thin hyphal sheath, projecting up to 30 µm above the hymenium, in upper quarter with few (1–4, rarely up to 8) low broadly conical teeth or protuberances up to 0.5(–1) µm long; cystidia and hyphidia absent; basidioles 12–15 x 3–4 µm; basidia subutriform, 15–35 x 4–5 µm, walls of basal part slightly thickened and encrusted with granules (as are the basidioles, too), with 4 thin sterigmata 3–4 µm long; spores broadly ellipsoid, with one side slightly flattened, 2.7–3.5(–4.0) x 1.8–2.5 µm (in African specimens 2.8–4.5 x 1.5–2.5 µm).

Causes white fibrous rot of wood.

> **Hawai'i.** F.L. Stevens 877 (BPI 278618) on frondose wood, identified by Burt as *H. spreta* (see Burt, 1923: 186); RLG 20978 on *Toona ciliata*, Stainback Hwy. **Kaua'i.** RLG 21190 on *Metrosideros polymorpha*, Lehua Puhi Trail, Koke'e St. Park. **Moloka'i.** RLG 23023 on *Araucaria heterophylla*, RLG 23026 on *Cupressus macrocarpa*, Kamakou Rd.

Comments. The very small protuberances (denticles) on upper part of setae are visible only when great magnification (x 1000 or more) is used. The similar species *H. tomentelloidea* has setae (35–)40–70 x 5–10 µm with 5–10 broadly conical teeth 0.8–1.5(–2) µm long and more elongated spores 3.1–4.5 µm long. *H. separabilis* is a species of Tropical African–Northwestern Pacific distribution and possibly of Gondwanan origin. The specimens found in Africa (Central African Republic, Gabon, Madagascar, Mauritius, Réunion) are all thin (about 60 µm), possibly young, and with better developed hyphal layer with loosely interwoven thin-walled hyphae (see Léger, 1998: 252–254).

Mean spore size and mean Q value of *H. separabilis*:

2.99 x 2.23	1.34	RLG 20978
3.17 x 2.24	1.41	RLG 23026

14. **HYMENOCHAETE SEPARATA** G. Cunn., Trans. Roy. Soc. New Zeal. 85 (1): 50. 1957.

Basidiomata perennial, effused, closely adnate, very hard when dry, as rounded patches 0.5–2 cm long, later confluent, 200–500, later up to 750 µm thick; hymenium coarsely low-tuberculate or smooth, with some crevices, dark Hazel to Fawn (M: 7.5 YR 5/4 or almost 7.5 YR 4/4; K & W: 7(–6) E , brownish terra cotta); margin abrupt, concolorous.

Tomentum, cortex and hyphal layer absent, setal layer sometimes indistinctly 2-layered.

Hyphal system subdimitic; skeletoids ascendant, with thickened brownish walls, 2.5–3.5 µm diam., densely agglutinated with brown resinous matter; generative hyphae few, subhyaline, with septa, 3–4 µm diam; setal layer and hymenium with numerous conglomerates of crystals 8–30 µm diam; setae numerous, broadly subulate, (30)35–45(–55) x 6–7(–8) µm, partly covered with a thin hyphal sheath, without incrustation or with a few crystals; cystidia and hyphidia absent; basidioles numerous, brownish, 22–28 µm long; spores (according to Léger, 1998: 255) ellipsoid, 5–6.5 x 3–3.5 µm.

> **Hawai'i.** RLG 20489, 23311 on *Coprosma montana*, RLG 23297 on *Pisonia sandwicensis*, Kipuka puaulu, HVNP.

Comments. All Hawaiian specimens are sterile; they may belong to one of the two Australasian species, *H. separata* or *H. vallata*. The last named species has spores about 4 x 2 µm and setae (23–)25–45(–55) x (4.5–)5–7.5 µm, but these are all covered with crystals. Setae of *H. separata* are bigger, described as (37–)45–55(–57) x (5.5–) 6–8 µm by Léger (1998: 255). *H. separata* has been found previously in New Zealand and in Réunion.

15. Hymenochaete subdissimilis *ad int.* — Figs. 1, *3*; 2, *6*

Basidiomata perennial, effused, closely adnate, hard when dry, detachable as small pieces, 100–400 µm thick; hymenium tuberculate, rather densely deeply cracked, Grayish Sepia (M: 5 YR 5/2; K & W: 7 (D–E) 3 – 8 E 3), margin abrupt (?).

Tomentum and cortex absent, hyphal layer 30–150 µm thick, pseudoparenchymatous, indistinctly different from the setal layer; setal layer thickening, not clearly stratose.

Hyphal system monomitic; hyphae very densely agglutinated with brown resinous matter, indistinct, 2–3.5 µm in diam, brown, thick-walled; crystalline matter present in context and hymenium, in context as conglomerates 15–50 µm in diam; setae numerous, broadly subulate, 40–60(–65) x 7–10 µm, projecting to 30 µm above the hymenium, with bluntly acute tip, straight, usually with a thin hyphal sheath, irregularly encrusted; cystidia and hyphidia absent; basidioles present; basidia subutriform, 12–16 x 4–5.5 µm, with 4 sterigmata 3–4 µm long; spores elongated-ellipsoid or short-cylindrical, slightly curved or with one side flattened, 3.7–4.4(–5) x 1.8–2.5 µm.

Causes white fibrous rot of wood.

> **Hawai'i.** RLG 19028 on *Psidium guajava*, Manuka State Park. **Maui.** RLG 16777 on *Syzygium jambos*, Mile 6, Hana Highway (identified in Gilbertson et al., 2002: 227 as *H. anomala* Burt).

Comments. This possibly new species is externally very similar to *H. dissimilis* as described by Léger (1998: 126–127) but differs in having a tuberculate hymenium, an indistinct hyphal layer, shorter setae and distinctly smaller spores. However, *H. dissimilis* described by Job (1990: 19–20) has setae similar to those in *H. subdissimilis* (45–65 x 6–8 µm). Another possibly closely related species, *H. unicolor*, differs in having a smooth (even) brown hymenium, smaller nonencrusted setae (30–)35–50 x 5–7 µm, basidioles with thickened walls at the base, and broader spores 3.4–4.8 x 2.2–2.8 µm.

Among specimens collected in Hawai'i, one (BPI 1103516, on *Cyathodes* sp., Maunahui, Moloka'i; Degener Otto 2823, 15 Apr 1928) is very similar to *H. subdissimilis* but has longer spores 4.2–5.2 x 1.7–2.4 µm (mean size: 4.80 x 2.08 µm, $Q = 2.31$). Its hymenium is Vinaceous Buff–Grayish Sepia (M: 2.5 YR 5.5/4). After more collections (including young and thin ones) are found, possibly all three species *(H. dissimilis, H. subdissimilis,* and *H. unicolor)* may be synonymous, and only one species with variable spore size exists.

Mean spore size and mean *Q* value of *H. subdissimilis*:

4.05 x 2.05	1.97	RLG 19028
4.19 x 2.16	1.94	RLG 16777

16. HYMENOCHAETE TOMENTELLOIDEA Gilb. & Hemmes, Mycotaxon 62: 476. 1997.

Figs. 1, *7*; 2, *1*

Basidiomata annual, effused, adnate, soft or coriaceous, when young 60–70 µm thick, then 200–450 µm, as rounded patches 0.5–2 cm in diam, later confluent and up to 10 cm long; hymenium even, in older parts irregularly cracked; grayish dark Cinnamon or Sienna-Umber (M: 7.5 YR 5–6/8 or 6/6; K & W: 6 D (6–7), 6 E 5 or 6 D 4); margin thin, indeterminate, lighter coloured than hymenium (sometimes M: 7.5 YR 6/10), slightly fibrillose, then distinct and concolorous with the hymenium.

Tomentum absent, cortex sometimes present but then indistinct, 20–40 µm thick; hyphal layer up to 300 µm thick; setal layer (when present) later thickening and up to 100 µm thick; dark line above the hymenium absent.

Hyphal system monomitic; hyphae brown, with thickened or thick walls, occasionally branching mainly at less than right angles, septate, 2–3.5 µm in diam, in hyphal layer loosely, then densely interwoven; setae numerous, subulate, with acute tip, (35–)40–70 x 5–7 µm, straight or some slightly curved, not encrusted, usually with a thin hyphal sheath, in upper third with few (5–10) broadly conical teeth 0.8–1.5(–2) µm long; projecting up to 20 µm above the hymenium; cystidia and hyphidia absent; basidioles 15–20 x 3.5–5 µm; basidia subutriform, 15–20 x (3.5–)4–5 µm, with lower part slightly thickened and encrusted with brown granules, with 4 thin sterigmata 3–4 µm long; spores ellipsoid, with one side slightly flattened, 3.1–4.5 x 1.8–2.5 µm.

Causes white fibrous or pocket (?) rot of wood.

HAWAI'I. RLG 20807 (holotype) on *Cibotium chamissoi*, Stainback Highway; RLG 18075, 18123 on *Metrosideros polymorpha*, Mile 18, Saddle Rd. **KAUA'I.** RLG 21106 on *Acacia koa*, Nualolo Trail, Koke'e State Park. **MOLOKA'I.** RLG 22952, on *Casuarina* sp., Kalaupapa Overlook.

Comments. The innumerable small denticles on upper part of setae are clearly visible only when great magnification (x 1000 or more) is used. Differences between this species and the very similar *H. separabilis* are described in the comments under that species. *H. separabilis* and *H. tomentelloidea* are possibly two closely related sibling species of Gondwanan origin, *H. tomentelloidea* being endemic.

Mean spore size and mean *Q* value of *H. tomentelloidea:*

3.40 x 2.14	1.59	RLG 18123
3.74 x 2.35	1.58	RLG 21106
4.11 x 2.43	1.69	RLG 18075
4.14 x 2.01	2.06	RLG 22952

17. HYMENOCHAETE UNICOLOR Berk. & M.A. Curtis, J. Linn. Soc., Bot. 10: 335. 1868.

Figs. 4, *11*; 2, *4*

Basidiomata perennial, effused, adnate, hard to woody when dry, when young 60–120 µm thick, when old up to 700 µm or more, as rounded patches 0.5–2 cm in diam, later confluent and up to 5 or more cm long; hymenium even, soon cracked, when old with deep cracks (fissures), Fulvous Umber or Sienna–Umber (M: 5 YR 4.5/6 to 5/4; K & W: 6 E 6), when old light grayish Umber (M: 5–7.5 YR 6/4; K & W: 6 (E–D) 4); margin distinctly delimited, lighter when young, soon abrupt and concolorous with the hymenium.

Tomentum and cortex absent; hyphal layer 30–50 µm thick, with some included setae, hardly distinguishable from the setal layer (with some setae), later disappearing; setal layer thickening, with indistinct layers; dark line above the hymenium absent.

Hyphal system monomitic; hyphae brownish, with thickened walls, scarcely septate, 2–3 µm in diam, in hyphal layer interwoven, in setal layer tightly agglutinated and encrusted with brown resinous matter; setae numerous, subulate, some slightly curved, with acute tip, (30–)35–50 x 5–7 µm, not encrusted, usually with a thin hyphal sheath, projecting up to 20 µm above the hymenium; hymenium composed of basidioles and basidia, no cystidia or hyphidia; basidioles almost cylindrical, with basal part thickened and encrusted with resinous matter (uneven), 15–25 x 3.5–5 µm; basidia subutriform, 15–25 x 4–5 µm, with 4 thin sterigmata; spores ellipsoidal with one side flattened or almost short-cylindrical, 3.4–4.8 x 2.2–2.8 µm.

Causes white fibrous rot of wood.

HAWAI'I. RLG 17718 on *Acacia koa,* Keanakolu Rd. KAUA'I. RLG 20601 on *Acacia koa,* Nualolo Trail, Koke'e State Park; RLG 21204 on *Myrica faya,* Lehua Puhi Trail, Koke'e State Park.

Comments. Identity of the specimens studied by us is not certain. Types of both *H. unicolor* and its synonym *H. lignosa* G. Cunn. are old thick specimens with only a few small spores, and most collections kept in herbaria under these names are sterile. Spores of the Hawaiian specimens are smaller than those described by Cunningham (1957), Reeves & Welden (1967), Job (1990) and Léger (1998), who all indicated the same (!) size, 4.5–5.5 x 3–3.5 µm. Asian and African specimens studied by Parmasto (2005) have spores 4.5–5.7 x 2.5–3.3 µm. The species complex including *H. unicolor, H. dissimilis, H. minuscula* and *H. subdissimilis* is in need of further study.

Mean spore size and mean *Q* value of *H. unicolor:*

3.65 x 2.33	1.56	RLG 20601
3.75 x 2.51	1.50	RLG 21204
4.41 x 2.32	1.90	RLG 17718

18. HYMENOCHAETE VAGINATA G. Cunn., Trans. Roy. Soc. New Zeal. 85 (1): 30. 1957.

Basidiomata effused, adnate but detachable as pieces, coriaceous, 120–300 µm thick, as rounded patches 0.5–1 cm long, then confluent; hymenium smooth (or tuberculate), Umber (M: 7.5 YR 4/6); margin Ochraceous (M: 7.5 YR 7/6–7).

Tomentum thin; cortex, hyphal and setal layers present but not easily distinguishable in young specimens; dark line above the hymenium absent.

Hyphal system monomitic; hyphae densely or almost loosely interwoven, with thickened walls, brownish, sparely branched, septate, 3–4 µm in diam; setae numerous, conical-subfusiform, 90–130 x 10–17(–20) µm, emerging up to 75 µm above the hymenium, with a hyphal sheath, not encrusted; hyphidia innumerous, brownish, with thickened walls, 2–3 µm in diam, some slightly flexuous; basidia subcylindrical, 18–25 x 4.5–5.5 µm, with 4 sterigmata; spores cylindrical, about 6.5–9.5 x 2.4–3.2 µm.

> O'AHU. C.L. Shear (BPI 1100612) on *Scaevola* sp., Pupukea Paumalu Forest Reserve, 15 Feb [1928 ?]. – **Isotype** studied: NEW ZEALAND, J.M. Dingley (K (M) 57642) on *Phyllocladus alpinus*, Wellington, Whakapapa, Mt. Ruapeku, 20 Oct 1949.

Comments. Until now the species was known only from a single New Zealand collection; its variation is unknown. The Hawaiian collection is a small young specimen but with big setae and spores characteristic for this species, and brownish hyphidia. According to Job (1991: 46) and Léger (1998: 288), the basidiomata of this species may be up to 800–1000 µm thick with thickening stratose setal layer. Isotype of this species is almost sterile with only few, partly collapsed spores (6–)6.8–8 x 2.4–3.2 µm (mean of 12 spores: 7.24 x 2.79 µm; *Q* = 2.60). The Hawaiian specimen has few cylindrical curved spores 7–9.5(–10) x 2.5–2.9 µm; mean of 14 spores: 8.37 x 2.64 µm, *Q* = 3.17.

Appendix

HYMENOCHAETE NOTHOFAGICOLA Parmasto, **sp. nova.** **Fig. 1,** ***13;*** **4,** ***2***

– *H. attenuata* sensu G. Cunn., Trans. R. Soc. New Zeal. 85 (1): 33. 1957.

> A H. attenuata *(Lév.) Lév. setis longis (55–)65–100 x (6–)7–11(–14) µm, sporis grandis (5.5–)6.0–8.0(–8.5) x (3–)3.5–4.5(–5.0) µm nec non pileis anguste reflexis ad 3 mm longis differt.*
>
> **Holotypus:** NEW ZEALAND, North Canterbury Distr., Arthur's Pass, 2500 ft, on *Nothofagus solandri,* Jan 1956, J.M. Dingley (PDD 16989).
>
> **Etymology:** *nothofagicola,* growing on *Nothofagus* spp.

Basidiomata effused-reflexed with narrow reflexed part (pileus) 1–3 mm broad, adnate but removable in pieces, soft coriaceous, 200–350 µm thick; pileal surface radially fibrillose or substrigose, then almost glabrous, with one or two concentric zones, brown (M: 5 YR 5/4–6; K & W: 6 (D–E) 5, Sahara); hymenium smooth but uneven, soon minutely radially or plumosely cracked, Fulvous or Sienna-Umber when young (M: 7.5 YR 5/8 to 5 YR 5/6; K & W: 6 D 7 to 6 D (4–5), raw Sienna, Sunburn or Camel), then Umber (M: 5 YR 4/5–6; K & W: 6 E (4–5), brown when old M: 5 YR 4/4); margin 0.5–1 mm broad, thin, sometimes torn, more lightly coloured (7.5 YR 7/10; K & W: 5 B 6, apricot yellow), soon concolorous with hymenium.

Tomentum present but in old specimens almost indistinct; cortex absent; context composed of hyphal layer gradually transiting to tomentum, and setal layer.

Tomentum 50–150 µm thick, hyphae loosely arranged; hyphal system subdimitic; setal hyphae absent; skeletoids 2–4 µm in diam, brownish, with thickened walls; generative hyphae yellowish, thin-walled, with rare septa; aggregates of crystals sometimes present in hyphal layer; setae numerous, (55–)65–100 x (6–)7–11(–14) µm, fusiform, with almost blunt or acute tip, straight or in upper part slightly curved, naked, in old specimens with hyphal sheaths, without incrustation; basidioles not numerous, similar to basidia; basidia broadly clavate, 18–25 x 6.5–8 µm, with 4 sterigmata; spores broadly ellipsoid, with one side flattened, or subcylindric, sometimes with a large guttule, (5.5–)6.0–8.0(–8.5) x (3–)3.5–4.5(–5.0) µm; in old specimens small crystals are present in hymenium.

Causes white fibrous rot of wood.

Specimens studied. Holotype (see above); **cotypes: New Zealand:** Taupo Distr., Kaimanawa Ranges, 2000 ft, on *Nothofagus solandri* var. *cliffortioides*, Dec 1946, G.H. Cunningham (PDD 4959); Kaimanawa Ranges, Upper Mohaka River, on *N. menziesii*, May 1953, J.M. Dingley (PDD 12542); same locality, on *N. fusca*, May 1953, J.M. Dingley (PDD 12593); Taupo Distr., Poronui, Upper Homestead, on *N. menziesii*, Jun 1953, J.M. Dingley (PDD 12596); Buller Distr., Totara Flats, Glandville Forest, on *N. fusca*, Apr 1955, J.M. Dingley (PDD 16595); all specimens determined by G.H. Cunningham as *H. attenuata* (Lév.) Lév.

Comments. This species was identified as *H. attenuata* by Cunningham. The true *H. attenuata,* which has not been found in Australasia, differs by densely parallelly compacted hyphae above the hymenium, shorter setae (35–)45–70(–80) µm long and small spores 3.5–5(–5.5) x (1.5–)1.7–2.2 µm. Description of *H. attenuata* by Job (1990: 7–8) combines the data on *H. attenuata, H. attenuata* sensu G. Cunn. (= *H. nothofagicola*) and a species collected in Switzerland and described by Job & Keller (1988).

Another species growing on *Nothofagus* spp. and closely related to *H. nothofagicola* is *H. australis* Parmasto & Gresl. which has been found in Southern South America where it is common in Tierra del Fuego. It differs in having a cortex and cylindrical spores 7–9 x 2.4–3.2 µm. The two sibling species possibly have common Gondwana origin. Both are superficially very similar to *H. tabacina*, which differs in presence of setal hyphae and smaller spores 4.2–6.8 x 1.3–2.2 µm.

Three of the specimens of *H. nothofagicola* studied by me are almost sterile, with a few damaged spores, the other three (including the holotype) have limited numbers of spores. In only one specimen (PDD 4959) was it possible to measure 25 spores. Their mean size is 7.23 x 4.28 µm; $Q = 1.69$.

Acknowledgments

Financial support from the Estonian Science Foundation (Grant no. 2145) to E. P. is gratefully acknowledged. He is thankful to the USA National Fungus Collections and New York Botanical Garden for the possibility to work in the herbariums (BPI, NY) in 1998 and 1999, and for loan of specimens from PDD. We thank Dr. Karen K. Nakasone and Dr. Don E. Hemmes who kindly reviewed the manuscript.

Literature cited

Bresadola J. 1915. Basidiomycetes Philippinenses (Series III). Hedwigia 56: 289–307.

Brummitt RK. 2001. World geographical scheme for recording plant distributions. Plant Taxonomic Database Standards No. 2, Ed. 2, August 2001. TDWG by the Hunt Institute for Botanical Documentation: Pittsburgh.

Burt EA. 1918. The *Thelephoraceae* of North America. X. *Hymenochaete.* Ann. Missouri Bot. Gard. 5: 301–372 + 2 pl.

Burt EA. 1923. Higher fungi of the Hawaiian Islands. Ann. Missouri Bot. Gard. 10: 179–189.

Carson HL, Clague DA. 1995. Geology and biogeography of the Hawaiian islands. In: Wagner WL, Funk VA (eds.), Hawaiian biogeography. Evolution on a hot spot archipelago. Smithsonian Institution Press: Washington & London, p. 14–29.

Cunningham GH. 1957. *Thelephoraceae* of New Zealand. XIV. The genus *Hymenochaete.* Trans. Roy. Soc. New Zeal. 85: 1–51.

Eldredge LG, Evenhuis NL. 2003. Hawaii's biodiversity: a reassessment of the number of species in the Hawaiian Islands. Supplement to the Records of the Hawaii Biological Survey for 2001–2002. Bishop Museum Occasional Paper 76: 1–28.

Gilbertson RL, Adaskaveg JE. 1993. Studies on wood-rotting Basidiomycetes of Hawaii. Mycotaxon 49: 369–397.

Gilbertson RL, Hemmes DE. 1997. Notes on fungi on Hawaiian tree ferns. Mycotaxon 62: 465–487.

Gilbertson RL, Bigelow DM, Hemmes DE, Desjardin DE. 2002. Annotated check list of wood-rotting Basidiomycetes of Hawai'i. Mycotaxon 82: 215–239.

Hino I, Katumoto K. 1964. Notes on some new species of fungi collected in the Ryukyu Archipelago. Bull. Fac. Agric. Yamaguti Univ. 15: 505–516.

Holmgren PK, Holmgren NH, Barnett LC (eds.) 1990. Index herbariorum. Part I: The herbaria of the World. 8th ed. New York Botanical Garden: Bronx, NY.

Job DJ. 1987. South African species of *Hymenochaete* (Aphyllophorales). South Afr. J. Bot. 53: 293–299.

Job DJ. 1991. Le genre *Hymenochaete* dans les zones tempérées de l'hémisphère sud. Mycol. Helvet. 1990, 4 (1): 1–51.

Kirk PM, Ansell AE. 1992. Authors of Fungal Names. Index of Fungi Supplement. Intern. Mycol. Inst.: Kew. 95 p.

Kornerup A, Wanscher JH. 1973. Methuen Handbook of Colour. 3rd Ed. London.

Léger J-C. 1998. Le genre *Hymenochaete* Léveillé. Bibliotheca Mycologica 171. J. Cramer: Berlin & Stuttgart. 319 p.

Munsell Book of Color. 1976. Baltimore.

Parmasto E. 2005. New data on rare species of *Hydnochaete* and *Hymenochaete (Hymenochaetales).* Mycotaxon 91: 137–163.

Rayner RW. 1970. A mycological Colour Chart. Commonwealth Mycological Institute and British Mycological Society: Kew.

Reeves F, Welden AL. 1967. West Indian species of *Hymenochaete.* Mycologia 59: 1034–1049.

MYCOTAXON

Volume 94, pp. 215–217 October–December 2005

A new predatory fungus from China

Dongshen Yang*, Weimin Chen*, Ying Huang,
Minghe Mo & Keqin Zhang

minghemo@yahoo.com.cn
Laboratory for Conservation and Utilization of Bioresources
Yunnan University, Kunming 650091, P.R. China

Abstract—*Dactylellina illaqueata*, a new predacious fungus capturing nematode by stalked adhesive knob in combination with non-constricting ring, is reported from Yunnan Province, China. The fungus is characterized by its simple, unbranched conidiophores singly bearing elongate fusiform conidia with 3-8 septa (usually 5) on the tip.

Key words—orbiliaceous fungi, nematode-trapping fungi

While surveying predacious fungi in Yunnan Province, soil samples were collected and spread on plates containing 2% corn meal agar medium. Nematodes (*Panagrellus redivivus*) were added in the plates as bait for predacious fungi. After 20 d for incubation at room temperature (about 20-28°C), predacious fungi were isolated under a dissecting microscope and identified according to the taxonomic system of Scholler et al. (1999). A new taxon, named *Dactylellina illaqueata*, is described here.

Dactylellina illaqueata D. S. Yang & M. H. Mo **sp. nov.** (Figures 1-15)

Mycelium effusum, hyphis sterilibus hyalinis, septatis, plerumque1.8-2.5 μm crassis. Conidiophoris hyalinis, septatis, erectis, simplicis, plerumque 95-250 μm altis, basi 2.2-2.6 μm crassis, apice1.8-2.1 μm crassis. Conidiis hyalinsi, elongato fusiformibus, apice rotundatis, basi truncates, 3-8 septatis (plerumque 5-septatis), 25.5-117.5 μm (saepe circa 66.5 μm) longis, 5.5-15.2 μm (saepe circa 4.8 μm) crassis. Chlamydosporis in culturis vetustioribus, globusis ad ellipsoidis.

Etymology: The species epithet refers to the species capturing nematodes by trapping devices.

Holotype: YMF1.01846D, Simao, Yunnan, China, Oct 2005, DongSheng Yang. The holotype and its living culture (YMF1.01846) were deposited in the Laboratory for Conservation and Utilization of Bio-resources, Yunnan, P. R. China.

Mycelium scanty, spreading, vegetative mycelium colorless, septate, mostly 1.8-2.5 μm wide. Conidiophores (Figs 1-3) colorless, erect, unbranched, often 95-250 μm high, 2.2-2.6 μm wide at base, and gradually tapering upward to a width of 1.8-2.1 μm at tip, bearing a single conidium on the tip, occasionally two conidia. Conidia (Figs 4-12) colorless, elongate fusiform, narrowly obtuse at the distal end, truncate at the base, the middle cell swelling obviously, 25.5-117.5(66.5)×5.5-15.2 (14.1) μm, 3-8 septa, mainly 5 septa. The proportion of conidia with 3,4, 5, 6, 7 and 8 septa accounts for 9.1%, 15.2%, 63.6%, 6.1%, 3.0% and 3.0%, respectively. When induced with nematodes, the fungus produced non-constricting ring (Fig 13) and stalked adhesive knob (Fig 14). Chlamydospores (Fig 15) spherical to ellipsoidal, intercalary.

---

* Authors DongShen Yang and WeiMin Chen contributed equally to this work.

Based on phylogenetic analysis of 18s rDNA, a new genus concept was proposed for predatory anamorphic *Orbiliaceae* by Scholler et al. (1999) in which the trapping device is the main morphological criterion for generic delimitation. In this taxonomic system, the genus *Dactylellina* M. Morelet emend. M. Scholler et al. includes three species capturing nematode by stalked adhesive knob in combination with non-constricting ring, *D. leptospora* (Drechsler) M. Morelet (Drechsler, 1937), *D. lysipaga* (Drechsler) M. Scholler et al. (Drechsler, 1937) and *D. yunnanensis* (K. Q. Zhang et al.) M. Scholler et al. (Zhang et al. 1996). *D. illaqueata* described here is mainly characterized by its 5-septate conidia singly bearing on the unbranched conidiophores and this species resembles *D. yunnanensis* and *D. lysipaga* in conidial shape. However, *D. yunnanensis* usually forms short denticles on tip of conidiophores and bears 2-5 conidia, and *D. lysipaga* produces the conidia mainly with 2-4 septa. In comparison with *D. leptospora*, the conidia of *D. illaqueata* usually have a wider middle cell (average 14.1 μm) than that of *D. leptospora* (4.0-5.8 μm). In addition, conidia of *D. leptospora* have more septa (5-15) than those of *D. illaqueata* (3-8, mainly 5 septa).

Acknowledgments

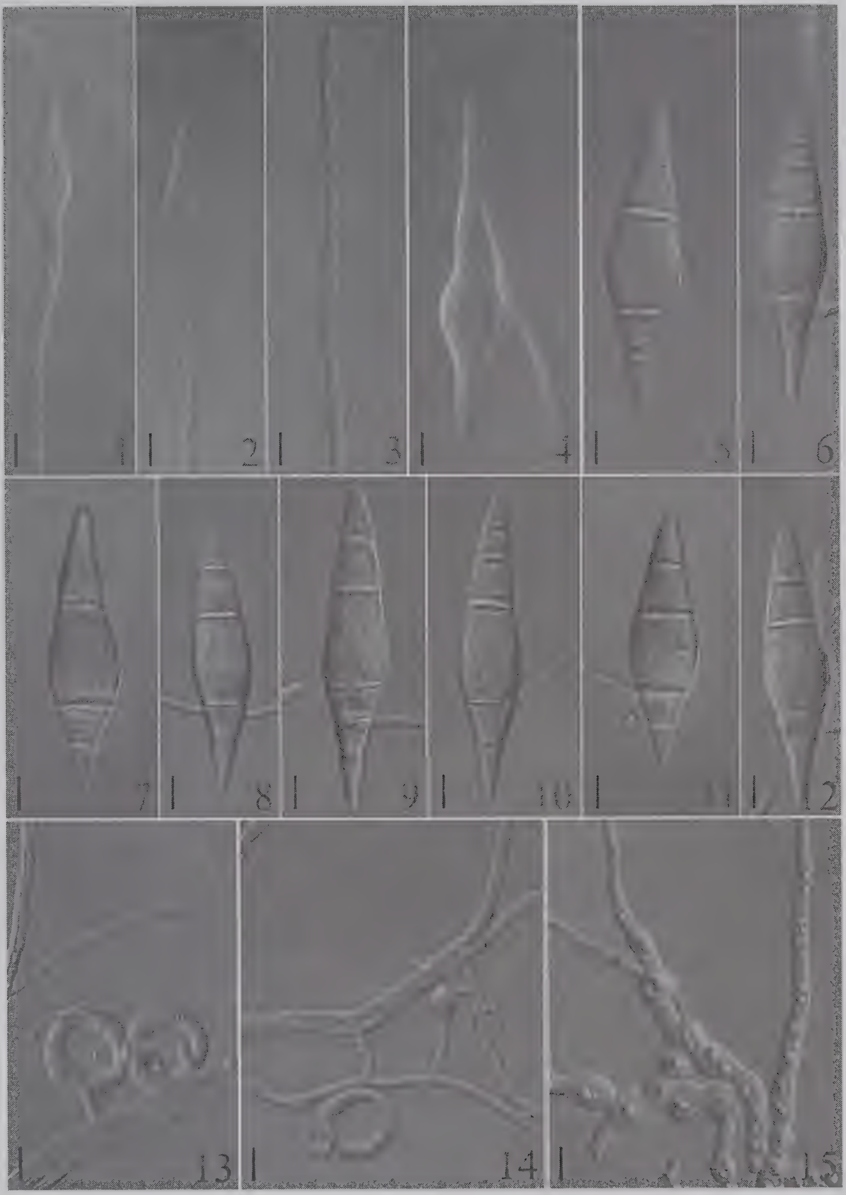
We are very grateful to the presubmission peer reviewers Dr. ShiDong Li and Dr. XueFeng Liu. We are also indebted to Prof. MeiHua Liu for helping with the Latin. This work was jointly supported by the projects from Ministry of Science and Technology of P. R. China (2003CB415102), NSFC (30460078), and Department of Science and Technology of Yunnan Province (2005NG05, 2003RC03, 2004C0001Q).

Literature Cited

Drechsler C. 1937. Some hyphomycetes that prey on free-living terricolous nematodes. Mycologia 29:447-552.

Scholler M, Hagedorn G, Rubner A. 1999. A reevaluation of predatory orbiliaceous fungi. II. A new generic concept. Sydowia 51(1): 89-113.

Zhang KQ, Liu XZ, Cao L. 1996. Nematophagous species of *Monacrosporium* from China. Mycol. Res. 100: 274-276.


1
2
3
4
5
6
7
8
9
10
11
12
13
14
15

Figs 1-15. *Dactylellina illaqueata*. 1-3. Conidiophores and immature conidia. 4. Immature conidia. 5-12. Mature conidia. 13. Non-constricting rings. 14. Adhesive knobs and non-constricting rings. 15. Chlamydospores. Bar=10μm.

MYCOTAXON

Volume 94, pp. 219–224 October–December 2005

New species of sterile crustose lichens from Australasia

John A. Elix

John.Elix@anu.edu.au
Department of Chemistry, Faculty of Science, Australian National University
Canberra, A.C.T. 0200, Australia

Abstract—*Hypocenomyce isidiosa* and *Lepraria lobata* from Australia, *Leprocaulon australasicum* from Australia and Norfolk Island and *Pertusaria cyathicola* from Norfolk Island are described as new to science. The species are distinguished by their characteristic chemistries and vegetative propagules.

Key words—chromatography, morphology, taxonomy

Introduction

Sterile crustose lichens are, in general, very poorly known although Tønsberg (1992), made a very significant contribution to the understanding of the Norwegian species. Like Tønsberg, I consider that the vegetative dispersal units (soredia, isidia, lobules, phyllidia and pseudopodetia) and the presence of diagnostic lichen substances to be important taxonomic characters. In this paper I describe four new species from Australia and Norfolk Island.

Materials and Methods

The morphology of the lichen specimens was examined using a Zeiss Stemi 2000C stereomicroscope, and a Zeiss Axiolab compound microscope. Chemical constituents were identified by thin layer chromatography (Elix & Ernst-Russell 1993), high performance liquid chromatography (Elix et al. 2003) and comparison with authentic samples.

Taxonomic Descriptions

Hypocenomyce isidiosa Elix, sp. nov.

Similis Hypocenomyce friesii *(Ach.) P. James & Gotth. Schneid. sed sterilis et superficie dense isidiatis differt.*

Etymology: The specific epithet is derived from the dense isidia characteristic of this species.

Type: **AUSTRALIA**. Western Australia, Charles Gardner Flora Reserve, central track, 20 km SW of Tammin along old York Road, 31°47'24"S, 117°28'07"E, 305 m, on dead, charred wood in *Eucalyptus* woodland with *Casuarina* and *Acacia* in shallow gully, *J. A. Elix 31849*, 22 Apr. 2004; holo: PERTH, iso-CANB.

KEY CHARACTERS — **Thallus** lignicolous, crustose to subsquamulose, scattered granules or squamules 0.1–1.2 mm wide, adnate, separate, not proliferating, green-brown, margin somewhat crenulate and ±upturned, squamules soon developing dense granular isidia which dominate the thallus, soredia absent. **Granular isidia** globose to subglobose, 0.03–0.05 mm high, with conspicuously blackened apices, becoming coralloid and densely crowded, forming cerebriform, conglomerate clusters, 0.5–2.0 mm wide. Apothecia and pycnidia not seen. **Chemistry** — K-, C-, KC-, Pd-, UV+ faint white; containing confriesiic acid (major), friesiic acid (minor).

Distribution — At present this species is only known from the type locality in Western Australia.

Ecology — This species is lignicolous on dead, charred *Eucalyptus* logs. Common associated species include *Flavoparmelia rutidota* (Hook. f. & Taylor) Hale, *Hertelidea pseudobotryosa* R.C. Harris et al., *Hypocenomyce australis* Timdal, *H. foveata* Timdal, *H. scalaris* (Ach.) M. Choisy, *Punctelia subalbicans* (Stirt.) D.J. Galloway & Elix and *Thysanothecium scutellatum* (Fr.) D.J. Galloway.

COMMENTS — *Hypocenomyce isidiosa* is a rare species characterized by the dominant, black-tipped granular, globose to subglobose isidia which coalesce to form cerebriform clusters largely obscuring the primary squamules and the presence of confriesiic and friesiic acids as lichen substances. Friesiic and confriesiic acids are very rare depsido-depsones so far only known from the genus *Hypocenomyce* (Elix et al. 2004; Timdal 1984). Both *Hypocenomyce friesii* and *H. caradocensis* (Leight. ex Nyl.) P. James & Gotth. Schneid. contain friesiic acid (major) and confriesiic acid (minor or trace). The new metabolite confreisiic acid shows an identical ultraviolet spectrum to freisiic acid, but appears to be a higher homologue with standard TLC R_F values: R_F (A) 0.12; R_F (B') 0.23; R_F (C) 0.16; standard HPLC RT=24.5 min. The present species is considered to belong to *Hypocenomyce* because of the unique chemistry and substrate preference, both of which are typical of this genus. Although common at the type locality, no fertile material of *H. isidiosa* was found. The squamules of *H. isidiosa* resemble those of *H. friesii* but are scattered and separate rather than being dense and contiguous to subimbricate. Furthermore, *H. friesii* differs chemically and lacks isidia.

Lepraria lobata Elix & Kalb, **sp. nov.**

Similis Lepraria jackii *Tønsberg sed thallo crassiore et lobato differt.*

Etymology: The specific epithet refers to the characteristic lobate margins of this species.

Type: **AUSTRALIA**. Western Australia, slopes of Angwin Peak, Porongurups Range, Porongurups National Park, 19 km ESE of Mt. Barker, 34°40'S, 117°51'E, 360 m, on granite rocks in low sclerophyll forest with heath and numerous granite outcrops, *J. A. Elix 41327, H. T. Lumbsch & H. Streimann*, 16 Sept. 1994; holo: PERTH.

KEY CHARACTERS —**Thallus** leprose-sorediate, granulose, whitish grey to greenish or bluish grey, usually delimited, forming extensive, irregularly spreading patches to 10 cm wide, or in small, irregularly roundish-colonies 0.5–1 cm wide that eventually coalesce; often with well-defined marginal lobes, 1–2 mm wide, ±raised at margins, covered with granules, thick (up to 250 µm), medulla white, distinct; hyphae 1.6–3 µm thick; soredia farinose, dispersed or forming a thick, continuous layer, ±roundish, 20–75 µm wide, commonly aggregated in roundish clumps (consoredia) up to a 350 µm wide, usually with short projecting hyphae to 20(–100) µm long; photobiont chlorococcoid, with individual cells 7–12 µm wide. Hypothallus not apparent. **Chemistry** — K+ yellow, C-, Pd+ pale yellow; containing atranorin (major), zeorin (major or minor, very rarely absent), rangiformic/jackinic acid (major) or roccellic/angardianic acid (major), ±norrangiformic/norjackinic acid (minor or trace), ±pallidic acid (minor), ±conpallidic acid (minor), ±3'-demethylatranorin (trace), ±3,7-di-*O*-methylstrepilin (trace), ±unknown dibenzofurans (minor), ±fragilin (trace), ±7-chloroemodin (trace), ±ursolic acid (minor).

Distribution — At present this species is known from Western Australia and the Australian Capital Territory.

Ecology — This species is corticolous on *Leptospermum* or muscicolous or terricolous in sheltered rock ledges. Common associated species on terricolous substrata include various *Cladonia* and *Xanthoparmelia* species, *Buellia substellulans* Zahlbr., *Lecanora farinacea* Fée and *Parmelia signifera* Nyl., and on corticolous substrata, *Candelariella xanthostigmoides* (Müll. Arg.) R. W. Rogers, *Flavoparmelia rutidota*, *Hypogymnia mundata* (Nyl.) Rass., *Parmelia pseudotenuirima* Gyeln., *Parmelina endoleuca* (Taylor) Hale, *Parmotrema reticulatum* (Taylor) M. Choisy and *Usnea inermis* Motyka.

COMMENTS — *Lepraria lobata* is a scattered species characterized by a relatively thick, grey-white, leprose thallus which typically becomes lobate at the margins and by the presence of atranorin, ± zeorin and fatty acids as the major lichen substances. Chemically *L. lobata* closely resembles *L. jackii* (Tønsberg 1992) in containing atranorin and fatty acids (i.e. jackinic/rangiformic acid, norjackinic/norrangiformic acid and/or roccellic, angardianic and toensbergianic acids) but is distinguished by usually containing high concentrations of zeorin (*L. jackii* rarely contains trace amounts of zeorin). Morphologically *L. lobata* differs from *L. jackii* in having a much thicker and compact thallus, a medullary layer and distinct, lobate margins. *Lepraria jackii* is normally unstratified and lacks lobate margins. *Lepraria borealis* Loht. & Tønsberg is similar to *L. lobata* since it forms lobed, rosette-forming thalli with a more or less granular upper surface and contains atranorin, rangiformic acid and ±roccellic acid. However, *L. borealis* never contains zeorin or pigments and the lobed margins are obscure.

SPECIMENS EXAMINED — **AUSTRALIA.** Australian Capital Territory, S of Paddys River near Murrays Corner, 35°24'S, 148°57'E, 550 m, on bark of *Leptospermum* in thicket, *J. A. Elix 647*, 13 Mar. 1975 (CANB). Western Australia, Gorge Rock, c. 24 km SE of Corrigin, 32°25'S, 118°00'E, 300 m, over moss on sheltered granite ledge, *K. & A. Kalb 35287*, 18 Aug. 1994 (CANB; herb. Kalb).

Leprocaulon australasicum Elix, **sp. nov.**

Similis Leprocaulon microscopicum *(Vill.) Gams ex D. Hawksw. sed superfice cinerascens et acidum protocetraricum, acidum norsticticum et acidum salazinicum continente differt.*

Etymology: The specific epithet is derived from the distribution of this species.

Type: **NORFOLK ISLAND**. Norfolk Island National Park, West Palm Glen Track, 29°01'06"S, 167°56'33"E, 140 m, on base of *Cyathea* in subtropical forest, *J. A. Elix 29042*, 16 June 1992; holo: CANB.

KEY CHARACTERS — **Primary thallus** crustose, arachnoid-tomentose, thin, persistent, white to off-white or pale cream, irregularly thickening, ±glabrous, becoming verruculose and somewhat leprose-granular, granules scattered, pale orange-brown, 20–40 µm wide. **Secondary thallus** of numerous, pale yellow-orange to orange-brown pseudopodetia, **pseudopodetia** globose at first but then elongate-cylindrical, delicate, fragile, simple or coralloid-branched and entangled, erect or ±decumbent, 0.1–1.0 mm high, 0.1–0.15 mm thick, bearing small, leprose-arachnoid granules, 20–70 µm wide, often with dense, projecting hyphae up to 20 µm long; photobiont chlorococcoid, with individual cells 7–10 µm wide. Hypothallus not apparent. Apothecia and pycnidia unknown. **Chemistry** — K+ yellow becoming dark red, C-, Pd+ orange-red; containing protocetraric acid (major), norstictic acid (minor), salazinic acid (minor), atranorin (minor).

Distribution — At present this species is known from three localities in subtropical rainforest in Queensland, New South Wales and Norfolk Island.

Ecology — This species is corticolous on the base of *Cyathea* or *Eucalyptus* or is lignicolous. Common associated species include various *Cladonia* species, *Cryptothecia bartlettii* G. Thor, *C. scripta* G. Thor, *Flavoparmelia euplecta* (Stirt.) Hale, *Lepraria atrotomentosa* Orange & Wolseley, *L. coriensis* (Hue) Sipman, *Parmotrema reticulatum*, *P. tinctorum* (Nyl.) Hale, and *Pseudocyphellaria poculifera* (Müll. Arg.) D.J. Galloway & P. James.

COMMENTS — *Leprocaulon australasicum* is a scattered species characterized by the conspicuous, pale yellow-orange to orange-brown pseudopodetia and the presence of protocetraric, salazinic and norstictic acids as the major lichen substances. Morphologically *L. australasicum* resembles *L. microscopicum* but the latter species differs in having a blue-green to yellow-grey upper surface with white pseudopodetia and in containing usnic acid, zeorin, ±atranorin, ±rangiformic acid (Lamb & Ward 1974). Furthermore, *L. microscopicum* grows on thin soil in rock crevices, peaty soils and only very rarely on the base of aged trees rather than predominantly on corticolous or lignicolous substrata.

SPECIMENS EXAMINED — **AUSTRALIA**. Queensland, Noosa Heads, Noosa National Park, along the Noosa Hill Track, 26°23'S, 153°06'E, 30 m, on base of *Eucalyptus* in coastal rainforest, *J. A. Elix 10369*, 31 July 1982 (CANB). New South Wales, Andersons Hill Road, Conglomerate State Forest, 21 km NW of Coffs Harbour, 30°04'S, 153°05'E, 240 m, on shaded rotting log in subtropical forest, *H. Streimann 60554*, 19 April 1998 (CANB). **NORFOLK ISLAND**. Norfolk Island National Park, track between Mt Pitt and Mt Bates, 29°00'50"S, 167°56'05"E, 270 m, on dead *Cyathea* in disturbed subtropical forest, *J. A. Elix 27367*, 15 June 1992 (B, CANB, NY); Norfolk Island National Park, West Palm Glen Track, 29°01'06"S, 167°56'33"E, 140 m, on base of *Cyathea* in subtropical forest, *J. A. Elix 29042*, 16 June 1992 (CANB); Pop Rock, near Mount Pitt Road, 29°01'23"S, 167°56'10"E, on dead *Cupressus* in disturbed subtropical forest, *J. A. Elix 29293*, 18 June 1992 (CANB); Norfolk Island National Park, Mount Pitt Road, 29°01'S, 167°56'E, 300 m, on treelet stem in disturbed subtropical forest, *H. Streimann 34843*, 10 Dec. 1984 (B, CANB).

Pertusaria cyathicola Elix, **sp. nov.**

Similis Pertusaria erythrella *Müll. Arg. sed arthothelinum et thuringionum continente differt.*

Etymology*:* The specific epithet derives from *Cyathea* and the Latin *cola* (dweller) in reference to the substrate of the holotype.

Type: **NORFOLK ISLAND**. Norfolk Island National Park, West Palm Glen Track, 29°01'06"S, 167°56'33"E, 140 m, on base of *Cyathea* in subtropical forest, *J. A. Elix 29043,* 16 June 1992; holo: CANB.

KEY CHARACTERS — **Thallus** crustose, off-white to pale greyish white, slightly cracked and areolate, surface slightly wrinkled and dull, verruculose, soon developing soredia, lacking isidia. **Soralia** yellow to yellow-orange, numerous, conspicuous, disc-like or sub-hemispherical, often constricted at the base, 0.5–2 mm diam., soredia farinose. Apothecia and pycnidia unknown. **Chemistry** — stictic acid (major), constictic acid (minor), arthothelin (minor), thuringione (minor), 3-*O*-methylthiophanic acid (trace), peristictic acid (trace), substictic acid (trace), hypostictic acid (trace), novostictic acid (trace).

Distribution — At present this species is only known from the type locality on Norfolk Island.

Ecology — This species is corticolous on *Cyathea.* Common associated species include various *Cladonia* species, *Cryptothecia bartlettii, Flavoparmelia euplecta, Lepraria atrotomentosa, L. coriensis, Leprocaulon australasicum, Parmotrema reticulatum, P. tinctorum* and *Pseudocyphellaria poculifera.*

COMMENTS — *Pertusaria cyathicola* is a rare species characterized by the conspicuous yellow to yellow-orange soralia and the presence of arthothelin, thuringione, stictic acid and constictic acid as the major lichen substances. Although no fertile specimens have been seen, this species has typical pertusarioid chemistry comprising the stictic acid chemosyndrome and xanthones. Morphologically *P. cyathicola* closely resembles *Pertusaria erythrella* but the latter species has white soralia and contains lichexanthone and the norstictic acid chemosyndrome (Archer 1997).

Acknowledgements

I thank Dr Alan W. Archer and Dr Patrick M. McCarthy for helpful discussions and amendments to the draft manuscript.

Literature Cited

Archer AW. 1997. The lichen genus *Pertusaria* in Australia. Bibliotheca Lichenologica 69: 1-249.

Elix JA, Ernst-Russell KD. 1993. A catalogue of standardized thin layer chromatographic data and biosynthetic relationships for lichen substances (2nd Edition). Australian National University, Canberra.

Elix JA, Giralt M, Wardlaw JH. 2003. New chloro-depsides from the lichen *Dimelaena radiata.* Bibliotheca Lichenologica 86: 1-7.

Elix JA, Tønsberg T, Wardlaw JH. 2004. The structure of friesiic acid, a novel lichen substance from *Hypocenomyce friesii.* Bibliotheca Lichenologica 88: 103-104.

Lamb, IM & Ward, A. 1974. A preliminary conspectus of the species attributed to the imperfect lichen genus *Leprocaulon* Nyl. Journal of the Hattori Botanical Laboratory 38: 499-553.

Timdal E. 1984. The genus *Hypocenomyce* (*Lecanolales*, *Lecideaceae*), with special emphasis on the Norwegian and Swedish species. Nordic Journal of Botany 4: 83-108.

Tønsberg T. 1992. The sorediate and isidiate, corticolous crustose lichens in Norway. Sommerfeldtia 14: 1-331.

MYCOTAXON

Volume 94, pp. 225–230 *October–December 2005*

Some interesting pyrenomycetous fungi on bark of *Quercus* spp. from Spain

JULIA CHECA & M.N. BLANCO

julia.checa@uah.es, natividad.blanco@uah.es

Dpto. Biología Vegetal. Univ
Alcalá de Henares. 28871 Alcalá de Henares, Madrid. Spain

Abstract—Four species of pyrenomycetous fungi (*Decaisnella mediterranea, Decaisnella mesascium, Ostreichnion nova-caesariense* and *Rosellinia tassiana*) growing on bark of *Quercus* spp were studied.

Keywords—*Pyrenulales,* Ascomycota

Introduction

During the study of pyrenomycetous fungi from the National Park of Cabañeros (Ciudad Real, Spain), the bark of *Quercus* spp. has been demonstrated as a very good substrate for the development of this kind of fungus.

This National Park is located in the centre of Spain and has one of the best representations of Mediterranean flora on the Iberian Peninsula. Here, there are forests of *Quercus faginea* Lam., *Q. ilex* ssp. *ballota* (Desf.) Samp., *Q. suber* L. and *Q. pyrenaica* Willd., accompanied by *Arbutus unedo* L., heaths and others shrubs. We have compared this area with a holm oak wood situated in Retiendas (Guadalajara, Spain) and found that both places share some interesting species, which are new records for Spain or Europe.

This paper deals with four species [*Decaisnella mediterranea, Decaisnella mesascium, Ostreichnion nova-caesariense* and *Rosellinia tassiana*] and provides new data on their areas and habitats.

Descriptions

Decaisnella mediterranea Checa & M.N.Blanco, Mycotaxon 91:353 (2005)

SPECIMENS EXAMINED— **SPAIN: Ciudad Real** NATIONAL PARK OF CABAÑEROS: 'EL CARACOL', 30.V.2003, bark of *Quercus faginea*, leg. J. Checa & M.N. Blanco, *AH 32203* (TYPUS). 30.IV.2004, *AH 33904* (PARATYPUS). 'EL LABRADILLO', 30.IV.2004, bark of *Quercus ilex* ssp. *ballota*, leg. J. Checa & M.N. Blanco, *AH 34188, 34186.*

Comments- The genus *Decaisnella* Fabre was reinstated by Barr (1979) who recognized two series of species on the basis of ascospore shape (Barr 1986).

D. mediterranea with fusiform ascospores, (12)-15-(17) transversal septa and 1-(2) longitunal septa, was described by us (Checa & Blanco 2005). It was found on *Quercus faginea* in the National Park of Cabañeros, and now we add other collections from *Quercus ilex* ssp. *ballota* in the same area.

Decaisnella mesascium (De Not.) M.E. Barr, *N. Amer. Flora*, Ser. 2, 13: 80 (1990)

FIGURE **1, A-B**

Ascomata scattered or clustered, erumpent to superficial, 0,5-1 mm diam., globose, papilla rounded, periphysate.

Asci clavate, with 8 biseriate ascospores, 185-215 x 35-40 µm; **pseudoparaphyses** trabeculate, 2 µm diam.; **ascospores** ellipsoid, 53-60-(64) x 17-22 µm, brown with pallid ends, verruculose, with 7-15 transverse septa and 2-3 longitudinal septa.

> SPECIMENS EXAMINED— **SPAIN: Ciudad Real** NATIONAL PARK OF CABAÑEROS: 'ARROYO BREZOSO', 21.XI.2003, bark of *Quercus faginea*, leg. J. Checa & M.N. Blanco, *AH 342224, 34225.* ibid. bark of *Quercus pyrenaica*, *AH* 34235. 'ARROYO MENGÜADOS', 30.IV.2004, bark of *Quercus faginea,* leg. J. Checa & M.N. Blanco, *AH 34202.* 'EL CARACOL', 8.XI.03, bark of *Quercus faginea*, leg. J. Checa & M.N. Blanco, *AH 34223*, 30.IV.2004, *AH 34210, 34226.* 'EL LABRADILLO', 30.IV.2004, bark of *Quercus ilex* ssp. *ballota*, leg. J. Checa & M.N. Blanco, *AH 34187*

Comments- Barr (1990) included *D. mesascium* in the group having oblong or ellipsoid ascospores with rounded or obtuse ends.

Although this species has been demonstrated to be very common on bark of *Quercus* spp., this is the first record in Mediterranean area; it seems to be chiefly European as Barr (1990) suggested.

Ostreichnion nova-caesariense (Ellis) M.E.Barr, Mycotaxon 3:84 (1975).

FIGURE **1, C-E**

Ascomata scattered, superficial, boat-like, 0,8-1,2 x 0,5-0,7 mm, with a longitudinal line of dehiscence.

Asci clavate, with 8 biseriate ascospores, 120-130 x 30-35 µm.; **pseudoparaphyses** trabeculate, in gel matrix; **ascospores** ellipsoid, 35-40 x 13-15 µm, sometimes constricted at the primary septum, 7-8-(13) transversal septa, Y-formed in the end cells and 1-3 longitudinal septa, brown, smooth or finely verruculose.

> SPECIMENS EXAMINED— **SPAIN: Ciudad Real** NATIONAL PARK OF CABAÑEROS: 'ARROYO BREZOSO', 21.XI.2003, bark of *Quercus faginea*, leg. J. Checa & M.N. Blanco, *AH 34236, 34237.* 'EL CARACOL', 30.V.2003, bark of *Quercus faginea*, leg. J. Checa & M.N. Blanco, *AH 32201, 32202.* 8.XI.2003, leg. J. Checa & M.N. Blanco, *AH 34238.* 30.IV.2004, *AH 34203, 34205, 34244, 34248.* 5-XI-2004, *AH 34213.* 'LAGUNA DE LOS CUATRO MORROS', 5.XI.2004, bark of *Quercus faginea*, leg. J. Checa & M.N. Blanco, *AH 34212.*

Comments- The genus *Ostreichnion* Duby is characterized by the conchate ascomata and muriform ascospores. It was created for two species, *O. europaeum* Duby and *O. americanum* Duby, the first one is a synonym of *Hysterium pulicare* Pers. and the second

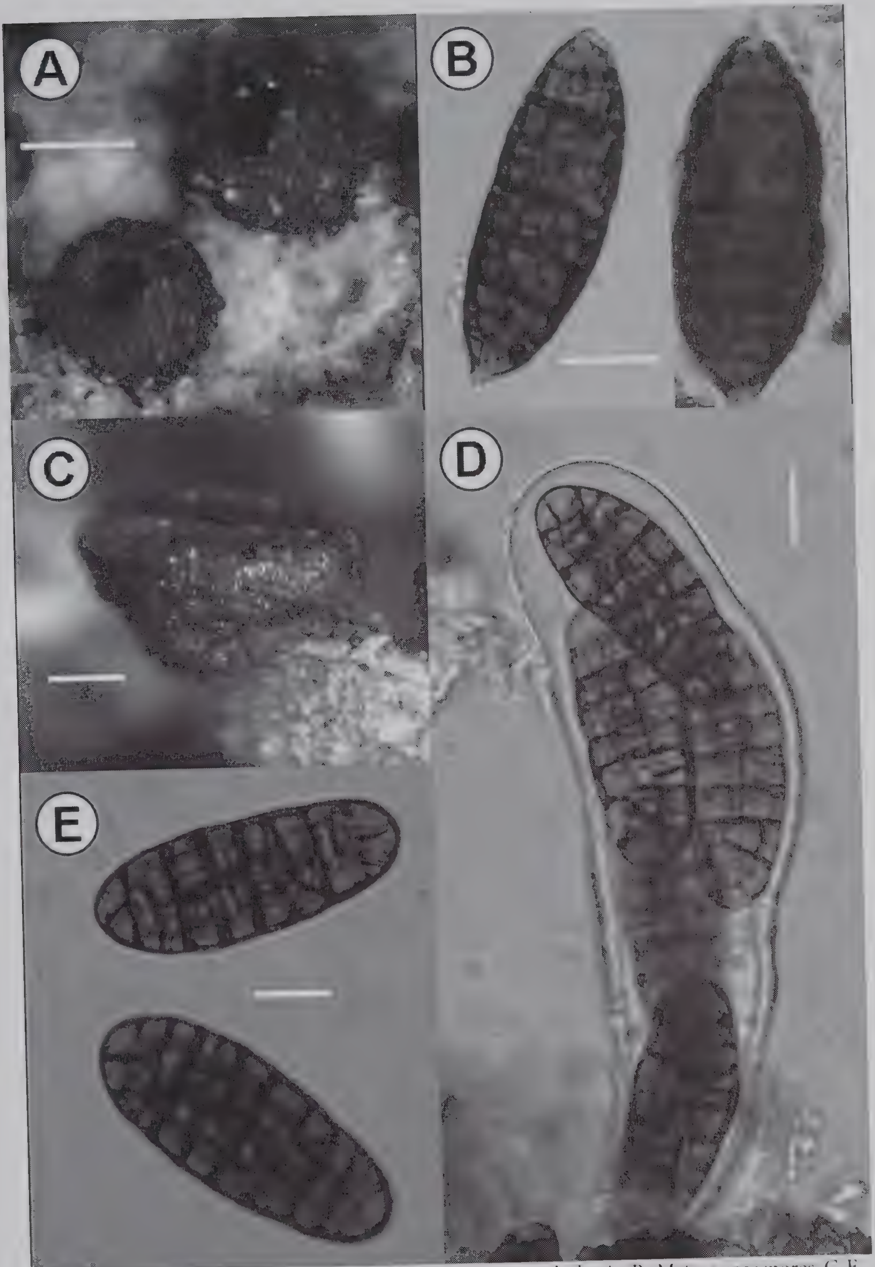


FIGURE 1.- A, B *Decaisnella mesascium*, (*AH 34223*). A.- pseudothecia. B.-Mature ascospores. C-E.- *Ostreichnion nova-caesariense* (*AH 34213*). C.- hysterothecia. D.- Ascus. E.- Ascospores. Scale bars. A,C- = 0,5 mm. B, D, E = 10 µm.

considered to be the same as *O. sassafras* (Schwein.) M.E.Barr. At present it includes three species: *O. curtisii* (Duby) M.E.Barr, *O. nova-caesariense* and *O. sassafras* (Barr 1975, 1990). These species have been treated in different genera of the *Hysteriaceae,* but the morphology of ascomata and ascospores justifies their inclusion in the *Mytilinidiaceae.*

O. nova-caesariense was regarded as a synonym of *Hysterographium flexuosum* (Schwein.) Sacc. (Zogg 1962), however there are some differences between the two genera. The conchate ascocarps, prosenchymatous peridium and trabeculate pseudoparaphyses characterize *O. nova-caesariense* as a member of the *Mytilinidiaceae.*

Barr (1990) indicated the bark of *Pinus rigida* as the substrate for this species, known only from type locality. We add some collections from *Quercus faginea* that provide a wider habitat and distribution of it. Probably, the substrate is not too important to this saprotrophic fungus (Barr, com. pers.).

Rosellinia tassiana Ces. & De Not., *Comm. Soc. crittog. Ital.* 1(4): 227 (1863)

FIGURE 2

Stromata scattered or clustered in groups of two or three from a common base, superficial, black, 1-1,5 mm diam., 2-2,5 mm long, subglobose, surface warty, with one papillate perithecium, 800-900 µm diam; ectostroma black, entostroma white.

Asci cylindric, 250-280 x 18-20 µm with 8 uniseriate ascospores; **paraphyses** 4-5 µm diam.; **ascospores** ellipsoid to ovoid, 30-37-(49) x 12-15 µm, dark brown, germ slit nearly spore length, straight, without appendages.

Cultures: Monosporic cultures were made in PDA, but they produced only sterile mycelium not the anamorph state.

SPECIMENS EXAMINED— SPAIN: Ciudad Real NATIONAL PARK OF CABAÑEROS: 'ARROYO BREZOSO', 6.XI.2004, bark of *Quercus ilex* ssp. *ballota*, leg. J. Checa & M.N. Blanco, *AH 34211*. **Guadalajara,** RETIENDAS: 30.I.2003, bark of *Quercus ilex* ssp. *ballota*, leg. J. Checa & M.N. Blanco, *AH 35129*. 12.II.2003, *AH 35125, 35126, 35127, 35128*.

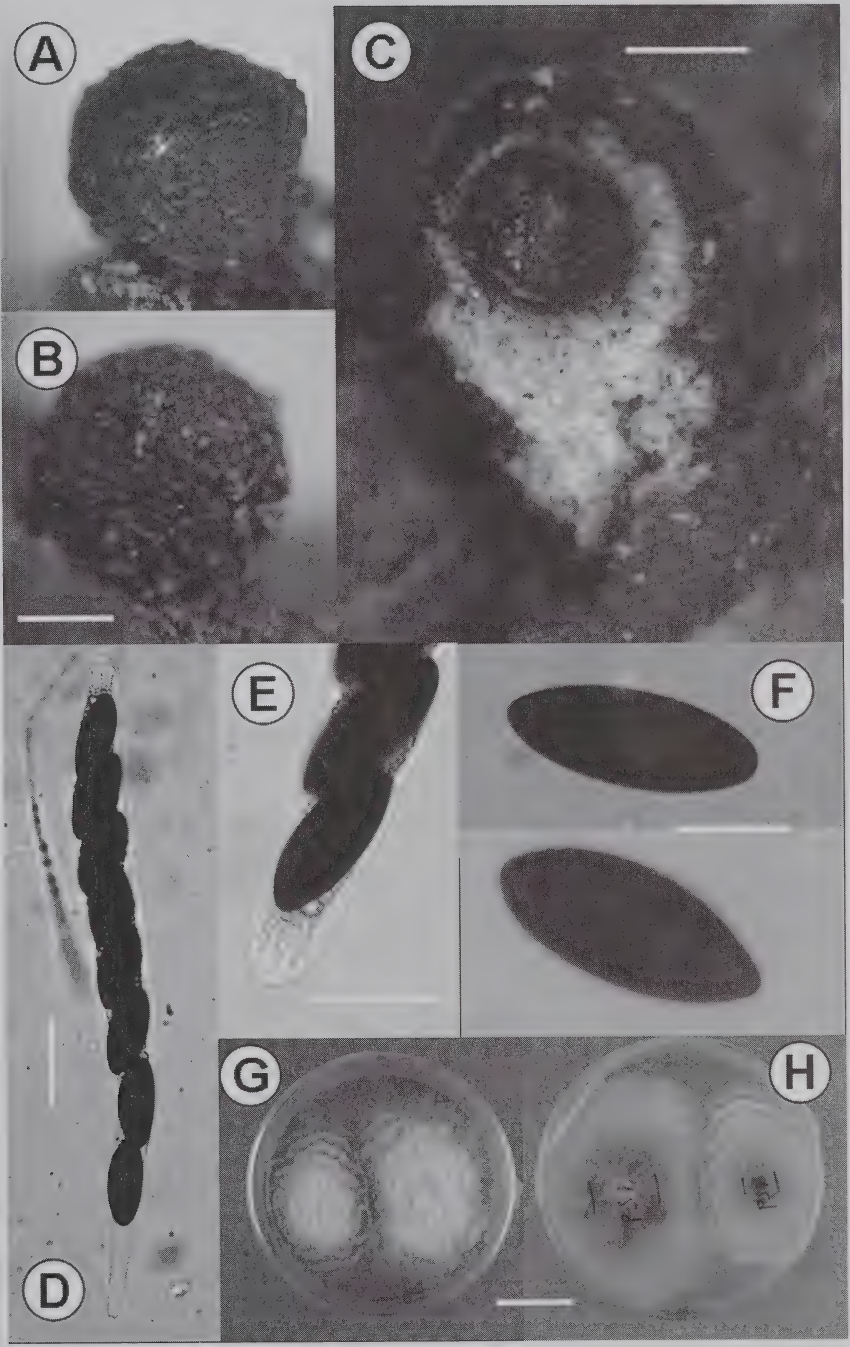
Comments- This species commonly grows on *Quercus* spp., but until now it was only known from Italy (Petrini 1993). This author considered *R. tassiana* to be related to uniperitheciate *Xylaria* or *Kretzschmaria* species and excluded it from *Rosellinia.*

The anamorph state is not known and our cultures from ascospores in PDA and MEA only produced sterile mycelium.

Acknowledgements

We want to express our gratitude to Dr. Margaret Barr and Dr. Larissa Vasilyeva for revision and expert criticism on the manuscript, and to the Project REN2002-01965.

FIGURE **2.**- *Rosellinia tassiana* (*AH 35127*) A, B.- Stromata. C.- longitudinal section of stroma. D.- Ascus. E.- Ascus apex. F.- Ascospores. G, H.- cultures; G- obverse; H- reverse.
Scale bars. A-C = 0,5 mm. D-E 35 µm. F = 15 µm. G-H = 2 cm.


A
B
C
D
E
F
G
H

Literature Cited

Barr ME. 1975. The genus *Ostreichnion*. Mycotaxon 3(1): 81-88.

Barr ME. 1979. A classification of Loculoascomycetes. Mycologia 71: 935-957.

Barr ME. 1986. On *Julella, Decalourea,* and *Decaisnella,* three dictyosporous genera described by J.H. Fabre. Sydowia 38:11-19.

Barr ME .1990. *Melanommatales* (Loculoascomycetes). North American Flora 2(3) : 1-129.

Checa, J. & M.N. Blanco. 2005. *Decaisnella mediterranea,* a new species on *Quercus faginea* from Spain. Mycotaxon 91:353-355.

Petrini 1993. *Rosellinia* species of the temperate zones. Sydowia 44:169-281.

Zogg, H. (1962). Die *Hysteriaceae* s. str. und *Lophiaceae.* Beitr. Kryptogamenfl. Schweiz. 11(3):1-190.

MYCOTAXON

Volume 94, pp. 231–234 *October–December 2005*

Biogeography and hosts of poroid wood decay fungi in North Carolina: species of *Fomes, Fomitopsis, Fomitella* and *Ganoderma*

L.F. Grand & C.S. Vernia

larry_grand@ncsu.edu
Department of Plant Pathology, North Carolina State University
Raleigh, North Carolina 27695-7616 USA

Abstract—Distribution and host species are given for two species of *Fomes*, one species of *Fomitella*, four species of *Fomitopsis* and five species of *Ganoderma*. A county distribution map is provided for eight species. Numerous new fungus-host plant associations are reported. Species checklist and figures can also be accessed at: http://www.cals.ncsu.edu/plantpath/people/faculty/grand/mycotaxon_4.pdf.

Key words—fungus distribution, polypores

Introduction

The importance of biodiversity and biogeography of fungi, especially in unique ecosystems and specific regions, was previously addressed by Grand & Vernia (2004ab, 2005). Studies by Jung (1987), Vernia & Grand (2000) and Grand & Vernia (2002, 2003) reported on the occurrence of host plants of poroid wood decay fungi in North Carolina. The distribution and host plants in North Carolina of species of *Phellinus* and *Schizopora* (Grand & Vernia 2004a), *Ceriporia, Ceriporiopsis* and *Perenniporia* (Grand & Vernia 2004b) and *Coltricia, Coltriciella* and *Inonotus* (Grand & Vernia 2005) were previously addressed. This report is the fourth in a continuing study of poroid wood-decay fungi in North Carolina and deals with species of *Fomes, Fomitella, Fomitopsis* and *Ganoderma.*

Materials and methods

Poroid wood-decay fungi were intensively collected in North Carolina over the past eight years (1997-2004). Collections, housed in the Mycological Herbarium, Department of Plant Pathology, North Carolina State University (NCSC), and records of the Plant Disease and Insect Clinic, Department of Plant Pathology, NCSU, were utilized in the results. Previous studies (Grand et al. 1975, Jung 1987) that contained data on county distributions were used in developing the distribution maps. Similarly, data from the BPI website (Farr et al. n.d.) provided some county data.

Collections were made of all species of *Fomes, Fomitella, Fomitopsis* and *Ganoderma* species on unusual hosts. Specimens were placed in paper bags in the field with a sample of decayed wood with most collections and field notes for all collections. Specimens were examined in the laboratory and identified using existing taxonomic treatments (Gilbertson & Ryvarden 1986, Jung 1987, Overholts 1953).

Nomenclature and authorities are from Gilbertson & Ryvarden (1986) and Index Fungorum (CABI Biosciences et al.) for the fungi and Kartesz (1994) for the host plant species.

The majority of collection sites were in state parks, gamelands and natural areas, Nantahala, Pisgah, Croatan and Uwharrie National Forests, the Blue Ridge Parkway and the Great Smoky Mountains National Park. A county distribution map is provided for all species that were recorded in three or more counties (Figs. 1–8).

Results and discussion

Fomes fomentarius (L. : Fr.) J. Kickx f. (Fig. 2) was found on six host species in 12 western counties, all in the Blue Ridge Mountains of the Southern Appalachian Mountain chain. *Fomes fasciatus* (Sw. : Fr.) Cooke (Fig. 1), a species with a distribution in the southern United States (Gilbertson & Ryvarden 1986), was collected for the first time in North Carolina, in three counties in the Coastal Plain and southern Piedmont regions. It appears that *F. fomentarius* reaches its southernmost distribution in the southern mountains of North Carolina and Tennessee and that *F. fasciatus* reaches its northernmost distribution in southern North Carolina.

Fomitopsis cajanderi (P. Karst.) Kotl. & Pouzar (Fig. 3) was found in 15 counties in the Blue Ridge Mountains and Piedmont regions and was recorded on five host species. *Fomitopsis pinicola* (Sw. : Fr.) P. Karst. (Fig. 4) was found in eight counties in the Blue Ridge Mountain region of western North Carolina.

Fomitella supina (Sw. : Fr.) Murrill, *Fomitopsis durescens* (Overh. ex J. Lowe) Gilb. & Ryvarden and *Fomitopsis spraguei* (Berk. & M.A. Curtis) Gilb. & Ryvarden were not collected frequently enough to determine any distributional patterns.

Five species of *Ganoderma* were recorded in this study. *Ganoderma applanatum* (Pers.) Pat. (Fig. 5) was found in 19 counties on 22 host species. *G. applanatum* is primarily distributed in the Blue Ridge Mountains of western North Carolina but collections were made in the eastern Piedmont and northern Coastal Plain regions as well. *Ganoderma tsugae* Murrill (Fig. 8), which is primarily found on *Tsuga canadensis* Carrière in North Carolina, was also found on *Abies fraseri* (Pursh) Poir. and *Pinus pungens* Lamb. *Tsuga caroliniana* Engelm. is most likely a host as well. Dead, needleless trees of *T. caroliniana* are difficult to distinguish from *T. canadensis. Ganoderma tsugae* is distributed in nine counties in the Blue Ridge Mountains in western North Carolina with a single report from a disjunct population of *T. canadensis* in the Piedmont.

Ganoderma lucidum (Curtis : Fr.) P. Karst. (*sensu lato*) is morphologically variable (Gilbertson & Ryvarden 1986) and considered by most taxonomists to be a species complex. With the exception of *Ganoderma curtisii* (Berk.) Murrill, *G. lucidum* was considered in the broad species concept in this study. *G. lucidum* (Fig. 7) is widely distributed in North Carolina and was found in 29 counties on 29 host species. The species concept of *G. curtisii* in this study was limited to those basidiocarps with a well-developed stipe, eccentric pileus and typically forming from underground roots near stumps. *G. curtisii* (Fig. 6) is widely distributed in North Carolina and was found in 11 counties on 14 host species.

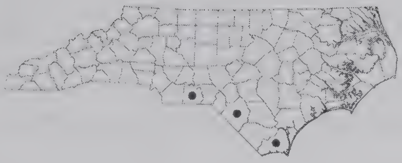


Fig. 1. Distribution of *Fomes fasciatus* in North Carolina.

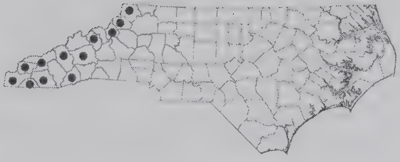


Fig. 2. Distribution of *F. fomentarius* in North Carolina.

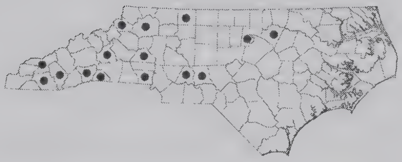


Fig. 3. Distribution of *Fomitopsis cajanderi* in North Carolina.

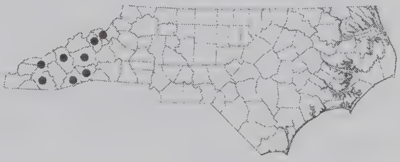


Fig. 4. Distribution of *F. pinicola* in North Carolina.

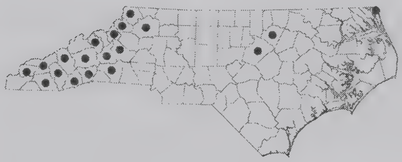


Fig. 5. Distribution of *Ganoderma applanatum* in North Carolina.

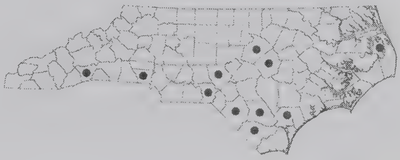


Fig. 6. Distribution of *G. curtisii* in North Carolina.

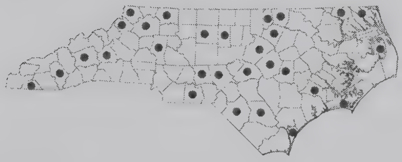


Fig. 7. Distribution of *G. lucidum* in North Carolina.

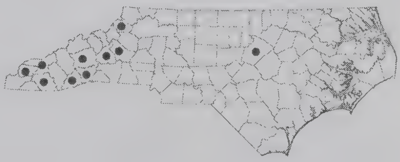


Fig. 8. Distribution of *G. tsugae* in North Carolina.

Acknowledgements

The authors thank Drs. Richard Baird and Lauraine Hawkins for suggestions and comments that improved the manuscript. Financial support for this project was provided, in part, by generous grants from the Highlands Biological Station (Highlands, NC). A special thanks to Tom Howard and the staff of the North Carolina State Parks system for permission to collect in the parks and natural areas of North Carolina. Richard Giles provided additional collections. Michael J. Munster assisted in the preparation of the manuscript.

Literature cited

Farr DF, Rossman AY, Palm ME, McCray EB. (n.d.) Fungal Databases, Systematic Botany & Mycology Laboratory, ARS, USDA [http://nt.ars-grin.gov/fungaldatabases/ (accessed on 04 October 2003)]

CABI Biosciences, Centraalbureau voor Schimmelcultures, Landcare Research (custodians). (n.d.) Index Fungorum [http://www.indexfungorum.org (accessed on 17 January 2006)].

Gilbertson RL, Ryvarden L. 1986. North American Polypores. Vol. 1. *Abortiporus – Lindtneria.* Fungiflora, Oslo. Pp. 1–433.

Grand LF, Menge JA, Bond JJ. 1975. Partial checklist of fungi from Highlands, North Carolina and vicinity. J. Elisha Mitchell Sci. Soc. 91:221–229.

Grand LF, Vernia CS. 2002. New taxa and hosts of poroid wood-decay fungi in North Carolina. Castanea 67:193–200.

Grand LF, Vernia CS. 2003. Noteworthy Collections, North Carolina, *Cryptoporus volvatus* (Peck) Shear. Castanea 68:88–89.

Grand LF, Vernia CS. 2004a. Biogeography and hosts of poroid wood decay fungi in North Carolina: species of *Phellinus* and *Schizopora*. Mycotaxon 89:181–184.

Grand LF, Vernia CS. 2004b. Biogeography and hosts of poroid wood decay fungi in North Carolina: species of *Ceriporia, Ceriporiopsis* and *Perenniporia.* Mycotaxon 90:307–310.

Grand LF, Vernia CS. 2005. Biogeography and hosts of poroid wood decay fungi in North Carolina: species of *Coltricia, Coltriciella* and *Inonotus*. Mycotaxon 91:35–38.

Jung HS. 1987. Wood-rotting *Aphyllophorales* of the southern Appalachian spruce-fir forest. Bibl. Mycol. 119:1–260.

Kartesz JT. 1994. A synonymized checklist of the vascular flora of the United States, Canada, and Greenland. Second Edition. Volume 1—Checklist. Timber Press, Portland, Oregon. 622 pp.

Overholts LO. 1953. The *Polyporaceae* of the United States, Alaska and Canada. University of Michigan Press, Ann Arbor. 466 pp.

Vernia CS, Grand LF. 2000. Polypores of a North Carolina Piedmont forest. Mycotaxon 74: 153–159.

MYCOTAXON

Volume 94, pp. 235–240 October–December 2005

Two new species of *Ramaria* from southwestern China

Ping Zhang

zhangping0000@163.net
College of Life Science, Hunan Normal University
Changsha 410081, Hunan Province, P. R. China

Zhu-Liang Yang

fungi@mail.kib.ac.cn
Kunming Institute of Botany, Chinese Academy of Sciences
Kunming 650204, Yunnan Province, P. R. China

Zai-Wei Ge

zwge@mail.kib.ac.cn
Kunming Institute of Botany, Chinese Academy of Sciences
Kunming 650204, Yunnan Province, P. R. China

Abstract—Two new species of the genus *Ramaria* collected from southwestern China are described and illustrated. They are *Ramaria luteoaeruginea* and *Ramaria pallidolilacina.* The former belongs to subgenus *Echinoramaria* while the latter to subgenus *Laeticolora.* Discussions on these two species and their affinities are furnished. The holotypes are deposited in the Cryptogamic Herbarium of Kunming Institute of Botany, the Chinese Academy of Sciences (HKAS).

Key words—clavarioid fungi, taxonomy

Introduction

Southwestern China, including Yunnan, Sichuan, Tibet and Guizhou, is rich in taxa of *Ramaria* Fr. ex Bonord. Several new species have been discovered and reported from this area previously (Petersen 1987a; Petersen & Zang 1986, 1989, 1990). While studying the *Ramaria* samples collected from the region, the authors have recently found two additional new species. They are reported herein.

Microscopic examinations were made with bright field and phase contrast optics. Measurements of spores were made in 5% KOH solution. The abbreviation [$n/m/p$] shall mean n basidiospores measured from m basidiocarps of p collections. Dimensions for basidiospores are given with notation of the form $(a)b–c(d)$. The range $b–c$ contains a minimum of 90% of the measured values. Extreme values, *i.e.*, a or d, are given in parentheses. Q is used to mean 'length/width ratio' of a basidiospore in side view; $\mathbf{Q}$ means average Q of all basidiospores ± sample standard deviation. For demonstration of spore ornamentation, aniline blue (cotton blue) staining was used following the method described by Kotlaba & Pouzar (1964). Color codes of the form "3B3" are from Kornerup & Wanscher (1981); Color names with first letter capitalized (e.g., Cream Color) are from Ridgway (1912).

Taxonomy

1. *Ramaria luteoaeruginea* P. Zhang & Zhu L. Yang, **sp. nov.** **Figs. 1-4**

Basidioma ramosum, ad 7 × 5 cm, obovatum ad obconicum;stipite ad 3 × 3 cm, crasso, singulari, albo, interdum aeruginescenti, cum ramulis abortivis, carne alba, non-gelatinosa; ramis crassis, luteis; apicibus acutis ad digitatis, aerugineis; basidiis 55–70 × 7–9 µm, clavatis, 4–sporigeris; sporis (9.5–) 10.5–12.5 (–13.0) × (4.5–) 5.0–6.0 (–6.5) µm, ellipsoideis vel elongato-lacrymiformibus, subechinatis. Fibulae praesentes.

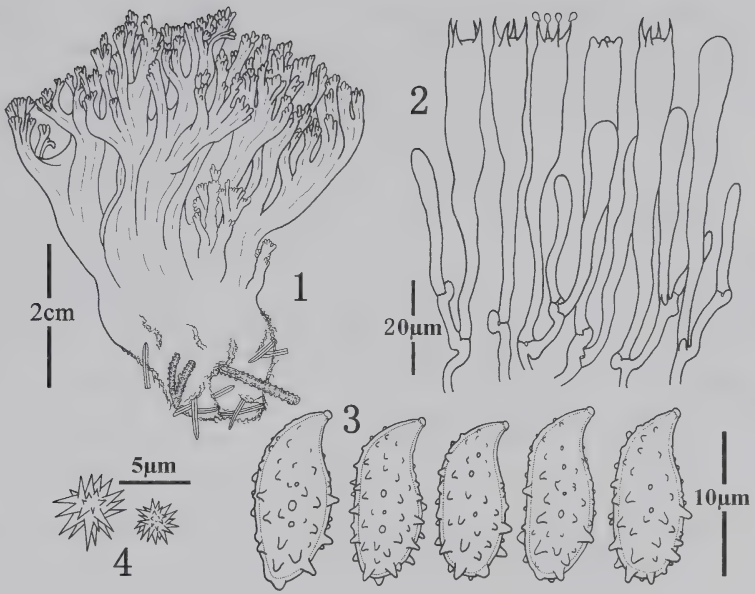
Holotype: Zhu L. Yang 4314 (HKAS 45693), 7. VIII. 2004, Changdu, Tibet, China.

Etymology: Referring to the colors of the yellow branches and more or less green apices.

Basidioma (Fig. 1) up to 7 cm high, up to 5 cm broad, repeatedly branched, usually obovate to obconic in outline. *Stipe* up to 3 × 3 cm, single or falsely fasciculate, smooth upward, tapering gradually downward into a tangle of white mycelia and slender rhizomorphs involving significant amounts of substrata, white [1A1], unchanging on drying, staining locally to intense verdigris [25C4-6, Montpellier Green, Light Porcelain Green] when fresh, remaining so after drying, with a few abortive stumps high on stipe; flesh white, solid, not gelatinous or slippery, dried flesh firm but easily penetrated. *Major branches* 4–6, stout, more or less terete, ascending, white below, upward concolorous with upper branches. *Branches* 4–5 ranks, ascending, polychotomous, longitudinally rugose; internodes diminishing gradually upward, buff-yellow [3A2-6, Straw Yellow, Warm Buff] below, gradually concolorous with apices; axils more or less rounded. *Apices* 1–2 mm long, acute to finger-like, dull blue-green [29D4-7, Dark Dull Yellow-Green]. *Taste and odor*, and *macrochemical reactions* not recorded.

Stipe tramal hyphae 2–8 µm wide, hyaline, thin-walled (wall up to 0.5 µm thick), parallel, clamped; ampulliform clamps occasional; gloeoplerous hyphae not observed. *Tramal hyphae of upper branches* 2–15 µm wide, hyaline, thin-walled, loosely parallel, clamped; ampulliform clamps occasional, up to 10 µm broad, thin-walled. Hyphae of basal mat 1.5–3.0 µm diam, thin-walled, clamped; stellate crystalline material (Fig. 4) often found between hyphae. *Subhymenium* rudimentary, hyphal. *Hymenium* thickening. *Basidia* (Fig. 2) 55–70 (–80) × 7–9 µm, clavate, clamped; contents homogeneous when young, becoming multiguttulate when mature, hyaline or slightly yellowish, slightly cyanophilous, 4-spored; sterigmata 4–6 µm long.

Basidiospores (Fig. 3) [40/2/1] (9.5–) 10.5–12.5 (–13.0) × (4.5–) 5.0–6.0 (–6.5) µm [Q = (1.90–) 2.00–2.36 (–2.40), **Q** = 2.19 ± 0.14], elongate pip-shaped, with apex mostly rounded, sometimes somewhat attenuate, with a distinct suprahilar depression, yellow-ocher under bright field; wall slightly thickened (up to 0.5 µm thick), moderately cyanophilous; apiculus not conspicuous, eccentric; ornamentation of numerous, scattered, strongly cyanophilius warts or obtuse spines up to 1 µm long.



Figs. 1–4: ***Ramaria luteoaeruginea*** **(HKAS 45693, holotype).**
1. Basidioma; 2. hymenium; 3. Basidiospores; 4. Crystal from basal mat.

Habitat: Gregarious on humus under *Picea*.

Known distribution: Only known from the type locality.

Notes: *Ramaria luteoaeruginea* is characterized by its stocky fruitbodies naturally staining blue-green both on stipes and apices in the field even without bruising, middle size spores [i.e., larger than those of *R. abietina* (Pers.: Fr.) Quél., but smaller than those of *R. grandis* (Peck) Corner], and subspinous spore ornamentation. It is a member of *Ramaria* subgenus *Echinoramaria* Corner (Corner 1950; Petersen 1981).

Ramaria luteoaeruginea is similar to *R. echinovirens* Corner et al., *R. glaucoaromatica* R.H. Petersen and *R. ochrochlora* Furrer-Ziogas & Schild. However, *R. echinovirens*, described from the Indian Himalayas, has orange yellow apices and was collected under *Quercus*. *Ramaria glaucoaromatica*, originally described from the Rockies, United States, can be separated from *R. luteoaeruginea* on its dull ocher apices and smaller spores (Petersen 1981). *Ramaria ochrochlora*, from the Alps in Switzerland, is distinguished from *R. luteoaeruginea* by its greenish yellow apices and smaller spores (Petersen 1981; Schild 1971).

These four species appear to be very closely related in having stocky fruitbodies, virescent color reactions and spinous or warty spores, and tending to occur in high elevation areas.

Singh (1977) reported a taxon from India, which is quite similar to *R. luteoaeruginea*. Branch tips of the specimens were noted as green. He identified the specimens as *R. ochrochlora*. The identification was dubious, because *R. ochrochlora* stained green only at the base, rather then on tips.

2. *Ramaria pallidolilacina* P. Zhang & Z. W. Ge, **sp. nov.** **Figs. 5-7**

Basidioma ramosum, ad 13 × 10 cm, obovatum ad subglobosum; stipite ad 3 × 2 cm, attenuato, singulari, albo ad cremeo, carne alba, non-gelatinosa; ramis pallide lilacinis, subrugulosis vel rugulosis, apicibus scopiformibus vel brevi-digitatis, cum ramulis concoloris; basidiis 60–85 × 9–11 µm, clavatis, 4-sporigeris; sporis (10.0–) 10.5–13.0 (–14.0) × (4.5–) 5.0–6.0 (–7.0) µm, ellipsoideis, irregulariter verruculosis. Fibulae absentes.

Holotype: Z. W. Ge 332 (HKAS 46112), 11. VIII. 2004, Leiwuqi, Tibet, China.

Etymology: Referring to the color of the branches.

Basidioma (Fig. 5) up to 13 cm high, up to 10 cm broad, repeatedly branched, usually obovate to subcircular in profile. Stipe up to 3 × 2 cm, single, tapering gradually downward, tomentose at base, smooth upward, without abortive branches, white to cream [1A1, 2A2], not changing color on bruising; flesh white, solid, not gelatinous or slippery. *Major branches* 4–6, stout, up to 1 cm thick, ascending, concolorous to branches above. *Branches* 4–5 ranks, ascending, more or less rugulose, polychotomous, pallid lilac [15A2-3; Light Vinaceous-Gray, Pale Light Vinaceous-Purple], slowly becoming purple-gray [14A2; Vinaceous-Gray] with spore deposit; internodes diminishing gradually upwards; axils more or less rounded. *Apices* obtuse, rather crowded, 0.5–1 mm long, molar-like when young, broom-form or short-digitate by maturity, concolorous with the branches. *Taste and odor*, and *macrochemical reactions* not recorded.

Tramal hyphae of stipe 3–15 µm wide, hyaline, thin-walled, clampless, interwoven; ampulliform septa and gloeoplerous hyphae not observed. *Tramal hyphae of upper branches* 3–10 µm wide, hyaline, thin-walled, loosely parallel, clampless; gloeoplerous hyphae not observed. *Subhymenium* rudimentary, hyphal. *Hymenium* unthickening. *Basidia* (Fig. 6) 60–85 × 9–11 µm, clavate, clampless, cyanophilous, 4-spored; sterigmata 6–8 µm long.

Basidiospores (Fig. 7) [60/2/2] (10.0–) 10.5–13.0 (–14.0) × (4.5–) 5.0–6.0 (–7.0) µm [Q = (1.62–) 1.85–2.40 (–2.60), **Q** = 2.14 ± 0.20], ellipsoid, more or less flattened adaxially, roughened in profile; wall up to 0.5 µm thick, cyanophilous; hilar appendix prominent; ornamentation of prominent, discrete, low warts and short ridges randomly placed.

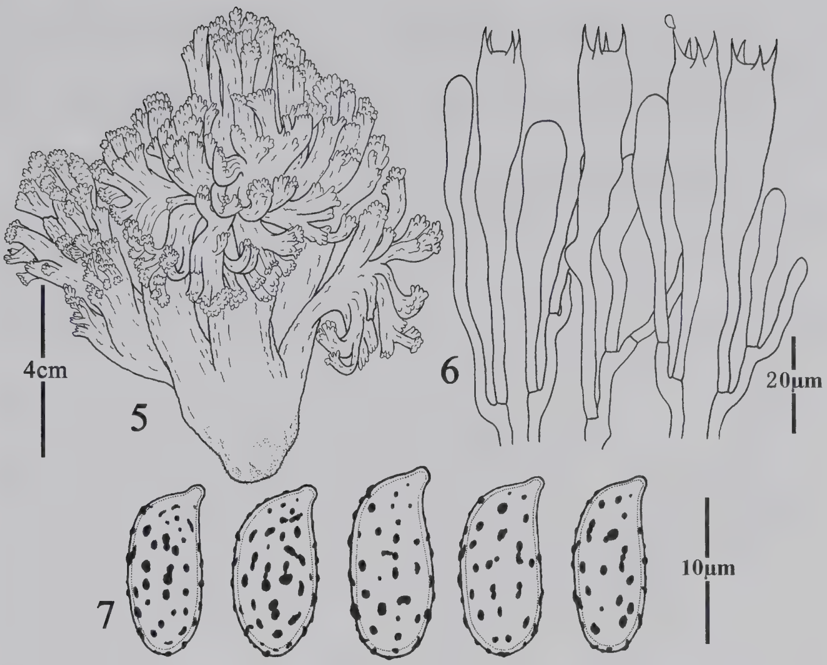
Habitat: Solitary on soil in *Picea* forest.

Known distribution: Only known from southwestern China.

Additional material examined: CHINA, Tibet, Jiangda County, near Tongpu village, alt. 3300m, 2-VIII-2004, Z. W. Ge 260 (HKAS 46040).

Notes: *Ramaria pallidolilacina* is characterized by its pallid lilac branches and apices, relatively small, white, tapering stipe, clampless basidia and coarsely ornamented spores. It belongs to *Ramaria* subgenus *Laeticolora* Marr & D. E. Stuntz (Marr & Stuntz 1973; Petersen 1987a, 1987b; Petersen & Zang 1989, 1990).

A group of taxa within *Ramaria* subgenus *Laeticolora* with lilac, violet or purple coloration has been described previously (Petersen 1987b). Several species included in this group such as *R. asiatica* (R.H. Petersen & M. Zang) R.H. Petersen, *R. cedretorum* (Maire) Malençon, *R. fennica* (P. Karst.) Ricken, *R. himalayensis* R.H. Petersen, *R. purpurissima* R.H. Petersen & Scates, *R. versatilis* Quél., can be easily separated from *R. pallidolilacina* by producing clamped basidia. Although *R. pallida* (Schaeff.) Ricken (=



Figs. 5–7: ***Ramaria pallidolilacina*** (HKAS 46112, holotype).
5. Basidioma; 6. hymenium; 7. Basidiospores.

R. mairei Donk) is a clampless species with purplish fruitbodies, its purple tints exhibit only at the apices. Furthermore, its stipe is more conspicuous than *R. pallidolilacina* (Petersen 1974). *Ramaria spinulosa* (Pers.: Fr.) Quél., reported from Europe and North America, with purple tinged fruitbody and clampless basidia, is also similar to *R. pallidolilacina*. But the former differs from the latter by its unique cinnamon tan stipe surface and pale beige stipe flesh (Petersen 1985; Schild 1990). *Ramaria subspinulosa* (Coker) Corner has smaller spores, and a tinge of lavender appearing only on the upper part of fruitbody when young (Coker 1923; Corner 1950; Petersen 1987b). *Ramaria fumosiavellanea* Marr & D. E. Stuntz, originally described from North America, with violet color component and clampless basidia, is distinguished from *R. pallidolilacina* by its darker (15-18D3) branches with grayish orange (5B3) apices and less ornamented spores (Marr & Stuntz 1973).

Acknowledgements

The first author is very grateful to Dr. R.H. Petersen, Tennessee, and Mr. E. Schild, Brienz, for their valuable published data on *Ramaria*. The authors thank Dr. R.H. Petersen and Dr. T.H. Li, Guangzhou, for their critical reviewing the manuscript. Dr. R. Kirschner, Frankfurt am Main, is acknowledged for his improvement of the Latin descriptions. Thanks are also due to Dr. W.Q. Hu, Montreal for his corrections of language. This study is supported by the National Natural Science Foundation of China (Nos. 30525002, 30420120049) and the NSF (DEB-0321846).

Literature cited

Coker WC. 1923. The Clavarias of the United States and Canada. The University of North Carolina Press: Chapel Hill (USA).

Corner EJH. 1950. A monograph of *Clavaria* and allied genera. Oxford University Press: London (UK).

Kornerup A, Wanscher JH. 1981. Taschenlexikon der Farben. 3. Aufl. Muster-Schmidt Verlag: Zürich (Switzerland).

Kotlaba F, Pouzar Z. 1964. Preliminary results on the staining of spores and other structures of Homobasidiomycetes in cotton blue and its importance for taxonomy. Feddes. Repert. 69: 131-142.

Marr CD, Stuntz DE. 1973. *Ramaria* in western Washington. Biblioth. Mycol. 38: 1-232.

Petersen RH. 1974. Contribution toward a monograph of *Ramaria*. I. Some classic species redescribed. Amer. J. Bot. 61: 739-748.

Petersen RH. 1981. *Ramaria* subgenus *Echinoramaria*. Biblioth. Mycol. 79: 1-261.

Petersen RH. 1985. Notes on clavarioid fungi. XX. New taxa and distributional records in *Clavulina* and *Ramaria*. Mycologia 77: 903-919.

Petersen RH. 1987a. A new species of *Ramaria* subg. *Laeticolora* from Yunnan. Acta Mycol. Sinica, Suppl. I: 255-257.

Petersen RH. 1987b. Contribution toward a monograph of *Ramaria*. VI. the *Ramaria fennica* - *versatilis* complex. Sydowia 40: 297-226.

Petersen RH, Zang M. 1986. New or interesting clavarioid fungi from Yunnan, China. Acta Bot. Yunnanica 8: 281-294.

Petersen RH, Zang M. 1989. *Ramaria* subgenera *Ramaria* and *Laeticolora* in Yunnan. Acta Bot. Yunnanica 11: 363-396.

Petersen RH, Zang M. 1990. *Ramaria* subgenera *Ramaria* and *Laeticolora* in Yunnan (continuation). Acta Bot. Yunnanica 12: 49-56.

Ridgway R. 1912. Color standards and color nomenclature. Washington, DC, Publ. by author.

Schild E. 1971. *Clavariales.* Fung. Rar. Icones Color. 5: 1-44.

Schild E. 1990. Studies in *Ramaria*. Z. Mykol. 56: 131-150.

Singh IP. 1977. Studies on clavarioid fungi of India. Ph.D. dissertation, Punjab University (India).

MYCOTAXON

Volume 94, pp. 241–244 October–December 2005

The world's second record of *Neoheteroceras flageoletii* reported from Turkey

Elşad Hüseyin[1], Faruk Selçuk[2], Ahmet Şahin[2]

elsadhuseyin@hotmail.com
[1]Gazi University, Kırşehir Arts and Sciences Faculty
Kırşehir, 40100, Turkey

[2]Fırat University, Arts and Sciences Faculty
Elazığ, 23100, Turkey

Abstract—*Neoheteroceras flageoletii*, a rare coelomycete with appendage-bearing conidia, is reported and illustrated from Turkey on *Tilia rubra* subsp. *caucasica*. It is a second locality of this fungus.

Key words— anamorphic fungi, morphology

Introduction

Fungi of Turkey have not been extensively investigated. Most studies research macromycetes, generally agaricoid fungi. Reports on micromycetes, including anamorphic fungi, were made by Bremer et al. (1947, 1952) and Petrak (1953). Data concerning the anamorphic fungi were first published as information on diseases of plants (Karel 1958) or fungal diversity investigations (Göbelez 1967). Anamorphic fungi of Turkey have been studied extensively, however, during the last ten years (Altan & Tamer 1996, Hüseyinov & Selçuk 1999, Braun et al. 2000, Hüseyinov 2000, Hüseyin & Selçuk 2001, Hüseyinov et al. 2002, Hüseyin et al. 2003, Selçuk et al. 2003, Mel'nik et al. 2004, Kırbağ 2004).

Material and Methods

The plant material for this paper was gathered from the forest of Rize province (Eastern Black Sea Region) in July 1998.

The host plant was identified according to Davis (1967). Specimens of the fungus were taken to the laboratory and microscopically examined under a Nikon compound microscope. Sections were hand cut using a razor blade. The fungus was identified following Nag Raj (1993). The specimen was deposited at GAZI.

Results

In this study, we clearly demonstrated that the fungus is a species of *Neoheteroceras*, viz. *N. flageoletii*. The description and illustration below is based on the Turkish material.

Neoheteroceras flageoletii Nag Raj, Coelomycetous anamorphs with appendage-bearing conidia, p. 539, 1993.

Conidiomata stromatic, subepidermal, innate-erumpent, broad conical in sectional view, 220–500 µm in diam in the base, uni- or bilocular, dark brown (Fig. 1A). **Conidiophores** cylindrical, simple, sparsely septate at the base, colourless. Paraphyses unbranched, filiform, septate, colourless, 50–65 x 2.5–3 µm. **Conidia** oblong to fusiform or stenoclavate, bearing appendages, 7– septate, faintly constricted at the septa, 45–58 x 7.5–10 µm including the apical and basal appendages; basal cell obconic with a truncate base, colourless, cellular appendage, 5–10 µm long; median cells dirty-brown to amber-brown 35–40 (–45) µm long, wall thick, smooth or slightly verruculose; apical cell conic, colourless, drawn out at the apex into an unbranched, attenuated, tubular appendage, 10–20 µm long; 1–2 sometimes 3 lateral appendages arising from the second, third and sixth median cells from the top, cellular, unbranched, colourless, sinuate, 7.5–22.5 x 2.5–3 µm (Fig. 1B).

Specimen examined—**TURKEY, Rize** Prov., Ardeshen district, near the Nursery of General Forest Manager, on dead branches of *Tilia rubra* DC. subsp. *caucasica* (Rupr.) V. Engler (*Tiliaceae*), 41°21'14"N, 41°13'50" E, 670 m, 30-VII-1998, Co. HUSEYIN E (EH 1281) and SELCUK F (FS 0206).

Discussion

The morphological characters of *Neoheteroceras flageoletii* of Turkish sample show some distinctions from the original collection. According to Nag Raj (1993) the fungus has conidia 48–60 x 7–11 µm, basal cellular appendage 3–8 µm long, apical cellular appendage 14–22 µm long and conidia with 2–3 lateral appendages, 12–30 x 2–3 µm. The fungus is terrestrial saprotroph, which requires a high level humidity and inhabits the bark of dead branches 0.5-1 cm diam.

Saccardo (1915) first described this fungus on *Tilia europaea* from France, as *Heteroceras flageoletii*. However, the monotypic genus *Heteroceras* Sacc. is an illegitimate later homonym, and consequently the species name is invalid. When Nag Raj (1993) re-examined the holotype, he published *Neoheteroceras* as a nom. nov. for the genus and *N. flageoletii* as a nom. nov. for the species. Until now there has been no additional information about this fungus. We think that our report from Turkey is the second registration of this fungus in the world.

The teleomorph of this species is unknown.

Acknowledgements

The authors would like to extend a sincere gratitude to Dr. Lorelei L Norvell (Mycotaxon) for linguistic help and a critique of the manuscript. We also thank Dr. V. Mel'nik (St. Petersburg) for kind help in identification of fungus, Dr. Cvetomir M. Denchev (Sofia, Bulgaria) and Dr. Tat'jana Andrianova (Kiev, Ukraine) for critically reading the manuscript and serving as presubmission reviewers.

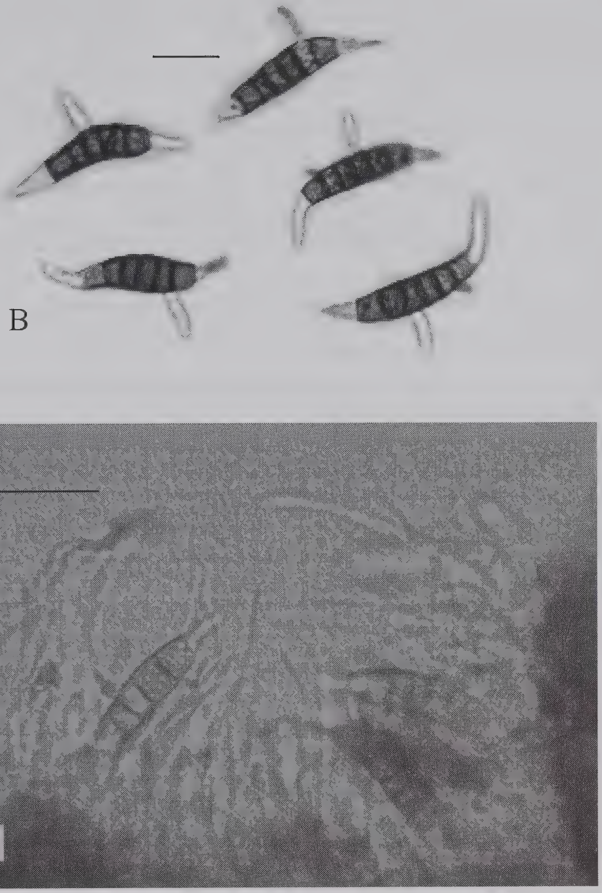


Fig.1. *Neoheteroceras flageoletii*: A.-vertical section of a conidioma. Scale bar=50 µm; B.-natural conidia. Scale bar=15 µm.

Literature Cited

Altan Y, Tamer AÜ. 1996. The parasitic fungi occurring on some endemic plants in Turkey and their damaging effects. Plant life in South-West and Central Asia. Ege University Pres, İzmir, Turkey, pp. 398-401.

Braun U, Mel'nik V, Hüseyinov E, Selçuk F. 2000. *Mycopappus alni* on species *Betula* and *Pyrus* from Turkey. Mikologia i Fitopatologia. "Nauka". St-Petersburg, 34(6): 1-2.

Bremer H, Ismen H, Karel G, Özkan M. 1947. Beiträge zur Kenntnis der parasitischen Pilze der Türkei. I. Revue de la Faculté des Sciences de I'Université d'İstanbul. Ser. B. 12(2): 307-334.

Bremer H, Karel G, Bıyıkoğlu K, Göksel N, Petrak F. 1952. Beiträge zur Kenntnis der parasitischen

Pilze der Türkei. VI. Revue de la Faculté des Sciences de I'Université d'İstanbul. Ser. B. 17(3): 259-275.

Davis PH. (Ed.). 1967. Flora of Turkey and East Aegean Islands, Vol. 2, Edinburgh Univ. Pres., Edinburgh. 581 pp.

Göbelez M. 1967. La Mycoflore de Turguie. II. Mycopathologia et Mycologia Applicata, 23(1): 47-67.

Hüseyin E, Selçuk F. 2001. New and poorly known genera of microfungi for Turkey, Turk. J. Bot., 25 (6): 437-438.

Hüseyin E, Selçuk F, Gaffaroğlu M. 2003. Some materials on mitosporic fungi from Turkey. I. *Hyphomycetes*. Botanica Lithuanica. Vilnius, 9(2): 151-160.

Hüseyinov E, 2000. New records of microfungi for Turkey. Israel Journal of Plant Sciences. 48(1): 75-78.

Hüseyinov E, Selçuk F. 1999. New records of phytopathogenic microfungi for Turkey. Plant Disease Research 14(2): 175-176.

Hüseyinov E, Mel'nik V, Selcuk F. 2002. *Ceratophorum helicosporum* – a new for mycoflora of Turkey genus and species of *Hyphomycetes* (*Dematiaceae*). Mikologia i Fitopatologia. "Nauka". St-Petersburg. 36(3): 11-13.

Karel GA. 1958. A Preliminary List of Plant Disease in Turkey. Ayyıldız Matb. Ankara. 44 pp.

Kırbağ S. 2004. New records of microfungi from Turkey. Pak. J. Bot. 36 (2): 445-448.

Mel'nik V, Hüseyin E, Selcuk F. 2004. Contribution to the studying of micromycetes in several Black Sea Provinces of Turkey. Academia Scientarum Rossica. Institutum Botanicum nomine V.L. Komarovii. Novitates Systematicae Plantarum non Vascularum. "Nauka", Petropolis, 37: 133-148.

Nag-Raj TR. 1993. Coelomycetous anamorphs with appendage-bearing conidia, Mycol. Publ., Waterloo, Ontario. 1101 pp.

Petrak F. 1953. Neue Beitrage zur Pilzflora der Turkei. Sydowia. Ann. Mycol. 7(I-4): 14-44.

Saccardo PA. 1915. Fungi noveboracenses. Ann. Mycol. 13: 115-138.

Selcuk F, Hüseyin E, Bitmiş K. 2003. Some materials on mitosporic fungi from Turkey. II. *Coelomycetes*. Botanica Lithuanica. Vilnius, 9(2): 161-170.

MYCOTAXON

Volume 94, pp. 245–248 October–December 2005

Lewia chlamidosporiformans sp. nov. from *Euphorbia heterophylla*

Bruno S. Vieira & Robert W. Barreto

rbarreto@ufv.br
Departamento de Fitopatologia, Universidade Federal de Viçosa
Viçosa, Minas Gerais, 36570-00, Brazil

Abstract—*Lewia chlamidosporiformans* found associated with the weed *Euphorbia heterophylla* (wild poinsettia) was collected in Brazil and is newly described and illustrated. It differs from the other species in the genus by having longer ascospores that are surrounded by a mucilaginous sheath and asci that have a different shape (obovoid to obpyriform). It produces abundant chlamydospores when grown in liquid media. Its pathogenicity to wild poinsettia was demonstrated.

Key words—*Pleosporaceae, Euphorbiaceae, Ascomycota*

Introduction

During isolations of fungal pathogens of weeds occurring in Brazil, cultures of a fungus showing typical features of members of *Lewia* (Simmons 1986a) were obtained from leaf spots on wild poinsettia (*Euphorbia heterophylla* L). *Alternaria*-like conidia were also produced. Members of *Alternaria* are known to be anamorphs of *Lewia*, helping to confirm that the fungus newly collected belonged to *Lewia*. This paper describes the teleomorphic stage of this fungus.

Although there are 50 known species of *Alternaria*, according to Kirk et al. (2001), only 5 species of *Lewia* have been described. Nevertheless, the total number or species presently included in this genus is 7 namely: *L. intercepta*, *L. infectoria*, *L. photistica*, *L. avenicola*, *L. ethzedia*, *L. sauropi*, *L. scrophulariae* (Simmons 1986a,b; Zhang & David, 1996; Simmons 2002; Kwasna & Kosiak 2003). Barreto & Evans (1998) provided a complete list of the fungal pathogens known in association with wild poinsettia but the list does not include any species of *Lewia* or *Pleospora* (the genera from which *Lewia* was segregated). There are also no recent reports of species in these genera associated with this host. A comparison of the fungus on wild poinsettia with the other species in the genus led to the conclusion that this represents a new species, which is described below.

Material and Methods

Cultures were obtained on VBA medium (vegetable broth-agar), a general-purpose medium (Pereira et al. 2003). Semi-permanent slides were prepared and mounted in lactophenol. Observations, measurements and photographs were made using an

Olympus BX 50 light microscope, fitted with a camera. Dry cultures of the fungus were prepared by following the methodology described by Rossman & Simmons (1999) and representative specimens of the fungus were deposited in the herbarium at the Universidade Federal de Viçosa (Herbarium VIC).

When grown in a liquid medium Jenkins-Prior (Fargues et al. 2001) under agitation at 180 rpm with a temperature of 25 ± 2 °C, the fungus formed abundant chlamydospores starting after 3 days of cultivation.

Confirmation of the pathogenicity of the isolate was made by utilizing a chlamydospore suspension, produced as described above, as inoculum. Inoculation of *E. heterophylla* plants was performed as follows: the concentration of the suspension was adjusted to 1 x 10^7 chlamydospores/ml with the help of a hemocytometer. Tests plants were brush-inoculated with the suspension on both sides of all leaves of the plants. After the inoculation, the plants were maintained in a dew chamber at 25 °C for 48 hours and later transferred to a greenhouse where they were observed daily for the appearance of symptoms. Plants brushed with sterile water served as controls. The experiment was accomplished with three replicates, each replicate consisting of a pot containing a 20-day old plant.

Taxonomic Description

Lewia chlamidosporiformans B.S. Vieira & R.W. Barreto, **sp. nov.** FIGS 1-3

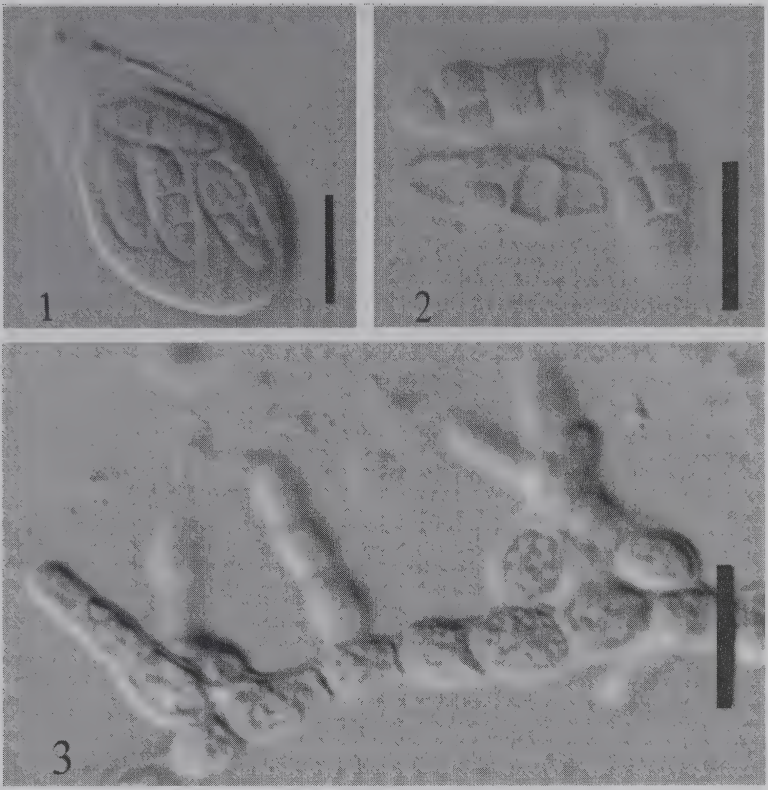
Ab Lewia photistica *Simmons; In VBA ascomata 145-190 x 115-160 μm, asci obovoidei vel obpiriformei, 80-120 x 37-44μm, ascosporae 25-37 x 10–12, cum tunica mucosa involutae, differens.*

Etymology: reference to the abundant formation of chlamydospores in liquid culture.

Holotype: on *Euphorbia heterophylla*, Brazil, Minas Gerais, Viçosa, January 2005, B.S. Vieira (VIC-28732).

Colonies on VBA (amended with 20 glucose g/L) 50 mm diam. after 7 days at 25 °C under a 12 hours photoperiod, cottony, grey, with some whitish areas, dark green reverse, with diurnal zonation with ascomata formation and sporulation. *Ascomata* pseudothecial, globose, dark brown, often immersed in the culture medium, 145-190 x 115-160 μm. *Asci* obovoid to obpyriform, thin-walled, bitunicate, 80-120 x 37-44 μm, 8 spored. *Ascospores* inordinate fusiform, often rounded at the apex and subacute at the base, usually constricted at the subapical transversal septum, 25-37 x 10-12 μm, subhyaline to very light brown, smooth, mature ascospores usually with 2 to 5 transverse septa and 1 or 2 longitudinal septa, surrounded by a distinct mucilaginous sheath. *Chlamydospores* formed abundantly in liquid culture media, in hyphae, intercalary, mostly in chains, 1-celled, subspherical, subcylindrical or irregular, 7 - 17 μm diam, thick walled, smooth, subhyaline to very light brown. *Anamorph*: a typical *Alternaria.*

Comments — Ascomata of *L. chlamidosporiformans* were produced *in vitro,* in synthetic media, namely: Kondryatiev medium and Goral medium (Fargues et al. 2001), submerged in those media, either singly or clustered. *L. chlamidosporiformans* resembles *L. avenicola* in this respect as described by Kwasna & Kosiak 2003 for ascomata produced on SNA slants. *L. sauropi* is also known to produce ascomata in culture (Zhang



Figs. 1–3. *Lewia chlamidosporiformans.* **Fig. 1.** Ascus with ascospores. **Fig. 2.** Mature ascospores (note the mucilaginous sheath surrounding the ascospores). **Fig.3.** Chain of chlamydospores with some undergoing germination. Bars = 24 µm (Fig. 1);32 µm (Fig. 2); 31 µm (Fig. 3)

& David, 1996). The other *Lewia* usually produce ascomata only on tissues of infected plants (Whitehead & Dickson 1952; Simmons 1986a,b). Sporadically, the formation of teleomorphs *in vitro* was observed by Bilgrami (1974) in *L. infectoria* (*Pleospora infectoria*) and Simmons (1986b) in *L. photistica.* Although *Alternaria* species and their teleomorphs may produce clumps of thick walled cells, they do not usually produce typical chlamydospores such as those of *L. chlamidosporiformans* (Ellis 1971; Ellis 1976). *L. avenicola* is known to produce chlamydospores arranged in chains or clusters on PDA and SNA (Kwasna & Kosiak 2003). *L. chlamidosporiformans* resembles *L. ethzedia* and *L scrophulariae* in ascomatal size (150-200 µm), but differs from the other species in this respect (Kwasna & Kosiak 2003). The sole species of *Lewia* known to attack a member of the *Euphorbiaceae* (namely *Sauropus androgynus* Merr.) is *L. sauropi.* This species differs from *L. chlamidosporiformans* in the following features: asci in *L. sauropi* have a different shape (obovoid, pyriform or ellipsoidal), asci are wider (47-57 µm) and no mention of chlamydospore formation was made in the original description (Zhang & David, 1996).

Three features are particularly useful for separating *L. chlamidosporiformans* from all other species in this genus, being unique for the new species: the ascus shape which is obovoidal to obpyriform (subcylindrical to subellipsoidal in all other species); the length of the ascospores is larger than described for other known species (25 -37 µm for *L. chlamidosporiformans* as compared to the the maximum length known for this genus until now *L. sauropi* - with a length of 29.5-35 µm); the presence of a mucilaginous sheath surrounding the ascospores (unknown for other members of *Lewia*).

Inoculation of *L. chlamidosporiformans* caused severe yellowing of leaves followed by a general necrosis and defoliation that finally led to plant death after 5 days of inoculation. Some diseased leaves were maintained in a dew chamber under a temperature of 25 °C for 24 hours and after this period abundant formation of conidia was observed.

Acknowledgments

The authors wish to thank Dr. Harry C. Evans and Dr. José Luiz Bezerra for reviewing the manuscript and to acknowledge the Conselho Nacional do Desenvolvimento Científico e Tecnológico (CNPq) for financial support.

Literature Cited

Barreto RW, Evans HC. 1998. Fungal pathogens of *Euphorbia heterophylla* and *E. hirta* in Brazil and their potential as weed biocontrol agents. Mycopathologia 141: 21-36.

Bilgrami RS. 1974. Occurrence of the perfect stage of *Alternaria tenuis* Nees on the leaves of *Marsilea quadrifolia* L. Current Science 43: 492.

Ellis MB. 1971. Dematiaceous Hyphomycetes. Commonwealth Mycological Institute, Kew.

Ellis MB. 1976. More Dematiaceous Hyphomycetes. Commonwealth Mycological Institute, Kew.

Fargues J, Smits N, Vidal C, Vey A, Vega F, Mercadier G, Quimby P. 2001. Effect of liquid culture media on morphology, growth, propagule production, and pathogenic activity of the Hyphomycete, *Metarhizium flavoviride*. Mycopathologia 154: 127-137.

Kirk PM, Cannon PF, David JC, Stalpers JA. 2001. Dictionary of the Fungi. CAB International, Wallingford, UK.

Kwasna H, Kosiak B. 2003. *Lewia avenicola* sp. nov. and its *Alternaria* anamorph from oat grain, with a key to the species of *Lewia*. Mycological Research 107 (3): 371-376.

Pereira JM, Barreto RW, Ellison CA, Maffia LA. 2003. *Corynespora cassiicola* f. sp. *lantanae*: a potential biocontrol agent for *Lantana camara* from Brazil. Biological Control 26: 21-31.

Rossman A, Simmons E. 1999. A modified technique for making dried cultures as voucher specimens. Inoculum. 50 (4): 3.

Simmons EG. 1986a. *Alternaria* themes and variations (14-16). Mycotaxon 25: 195-202.

Simmons EG. 1986b. *Alternaria* themes and variations (22-26). Mycotaxon 25: 287-308.

Simmons EG. 2002. *Alternaria* themes and variations (305-309) *Lewia/Alternaria* revisited. Mycotaxon 83: 127-145.

Whitehead MD, Dickson JG. 1952. Pathology, morphology and nuclear cycle of two new species of *Pyrenophora*. Mycologia 44: 747-758.

Zhang T, David JC. 1996. Notes on *Alternaria* species on *Euphorbiaceae* in IMI and K. I. Taxa on *Euphorbia, Acalypha* and *Sauropus*. Mycosystema 8-9: 109-121.

MYCOTAXON

Volume 94, pp. 249–252 October–December 2005

Weddellomyces turcicus, a new species on a grey *Acarospora* from Turkey

M. Gökhan Halici*, Alan Orange**, Ahmet Aksoy***

*mghalici@erciyes.edu.tr ***aksoy@erciyes.edu.tr
Erciyes Universitesi, Fen Edebiyat Fakültesi, Biyoloji Bölümü
38039 Kayseri, TURKEY

**alan.orange@nmgw.ac.uk
Department of Biodiversity and Systematic Biology, National Museum of Wales
Cardiff CF10 3NP, U.K.

Abstract—*Weddellomyces turcicus* is described as new from the thallus of an unidentified grey placodioid Acarospora in Turkey; it differs from other species of the genus in the asci being 2-spored when mature.

Key words—Ascomycetes, cephalothecoid exciple, lichenicolous fungi, *Dacampiaceae*

Introduction

The genus *Weddellomyces* comprises 11 species occurring on saxicolous lichens. The species have been treated by Calatayud & Navarro-Rosinés (1998) and Navarro-Rosinés & Roux (1995). The perithecial wall in this genus is composed of cephalothecoid plates, but Alstrup & Hawksworth (1990) also included one species without such plates. A recent collection from Turkey differs significantly from previously described taxa, and is described here as a new species.

Methods

Sections were prepared by hand, and examined in Congo Red (1% solution in water, mixed 1: 1 with 10% KOH), I (Lugol's Iodine: I 0.5 g, KI 1.5 g, water 100 ml), and Brilliant Cresyl Blue in water. The pigment of the ascomatal wall was investigated following the methods of Meyer & Printzen (2000).

The species

Weddellomyces turcicus Halici & Orange **sp. nov.** **Figs. 1–2**

> *Fungus lichenicola. Ascomata globosa, 390–490 μm diam., nigra. Excipulum superum ex segmentis cephalothecoideis constructum. Hamathecium ex hyphis ramosis, anastomosantibus et septatis compositum. Asci maturi 2-spori. Ascosporae brunneae, 3-septatae, 50–61(-69) × (16.5-)18.5–21.5(-22) μm, verruculosae.*

Typus: Turkey, Kayseri, Aladaglar Milli Parki, Hacer Ormanlari, 37°47'N, 35°18'E, alt. 1648 m, on thallus of *Acarospora* sp. on exposed limestone rocks, 20 November 2004, M.G. Halici 0.2018 (NMW C.2005.013.1–holotypus).

Lichenicolous, on dead and decolourized areas of the host thallus. Ascomata perithecioid, immersed in host thallus, erumpent, the apex visible through an irregularly shaped split in the host cortex, later the upper half of the perithecium exposed by the break up of the dead host tissue; ascomata globose, 390–490 μm diam., black, matt, slightly roughened, without setae, apex opening by a minute irregularly shaped depression, often with a few irregular radiating cracks. Exciple in surface view of polygonal cephalothecoid plates 30–105 × 30–50 μm, comprising dark-pigmented areas separated by pale areas, plates becoming indistinct in the lower part of exciple; in vertical section exciple 24–40 μm wide at sides and base, 40–60 μm thick near apex; cells slightly tangentially compressed, mostly 5.5–16.5 × 4–15 μm, the walls 1–2.5 μm thick, outermost layers of cells dark-pigmented except at the junctions of the plates, pigment within cell wall, unevenly distributed in inner cells of the pigmented layer, obscuring cell outlines in outer cells; pigment dark (slightly reddish) brown in water, K + dark greyish brown, HCl + reddish brown; after treatment with N, the colour in K is similar to the colour in water (Atra-brown). Subhymenium c. 20 μm thick. Hamathecium of richly branched and anastomosing interascal filaments 2–3.5 μm wide, with frequent septa. Asci cylindrical, 140–160 × 23–27 μm, initially up to 8-spored, but 2-spored at maturity, wall gradually thickened towards apex, I –, ocular chamber present. Ascospores (2–)3-septate, 50–55.6–61(–69) × (16.5–)18.5–19.9–21.5(–22) μm, length/breadth ratio (2.3–)2.6–2.8–3.0(–3.2) [n = 33]; brown, terminal cells concolorous or very slightly paler than central cells; surface verruculose, perispore not detected in mature spores, torus inconspicuous at all stages; wall of semi-mature to mature spores I + dilute blue (partly masked by brown pigment).

Discussion

The species of *Weddellomyces* are distinguished mainly by features of the ascospores, including size, shape, septation, and surface ornamentation; and by the identity of the host. *Weddellomyces turcicus* differs from all other members of the genus in the asci 2-spored at maturity, although the asci initially have 6-7 ascospores visible, and it is likely that 8 are present. It is also the first species in the genus to be recorded from *Acarospora*. It is known only from the type collection. *W. turcicus* is likely to be pathogenic, as it occurs on two small, bleached areas of host thallus, but further collections are necessary to confirm this. The currently unidentified host has a grey, placodioid thallus which is K + yellow, C –.

Acknowledgments

We would like to thank Prof. David L. Hawksworth and Dr Paul Diederich for reviewing this paper and for their helpful comments.

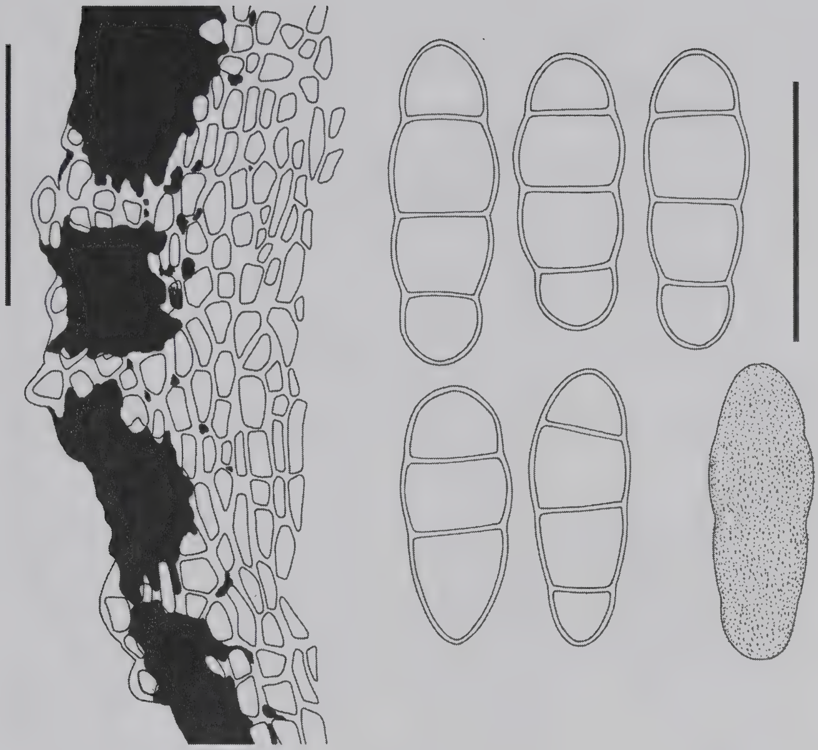


Fig. 1 – *Weddellomyces turcicus* (Holotype). Line drawings. a. section of part of upper exciple, showing parts of four cephalothecoid plates. b. ascospores (outlines). c. ascospore, showing surface ornament. Bar = 50 µm.

References

Alstrup V, Hawksworth DL. 1990. The lichenicolous fungi of Greenland. Meddel. Grønland, Bioscience **31:** 1–90.

Calatayud V, Navarro-Rosinés P. 1998. *Weddellomyces xanthoparmeliae* sp. nov. and additions to the chorology of other species of the genus. Mycotaxon **69:** 503-514.

Meyer B, Printzen C. 2000. Proposal for a standardized nomenclature and characterization of insoluble lichen pigments. Lichenologist **32:** 571-583.

Navarro-Rosinés P, Roux C. 1995. Le genre Weddellomyces (Dothideales, Dacampiaceae) en Catalogne et en Provence. Mycotaxon **53:** 161-187.

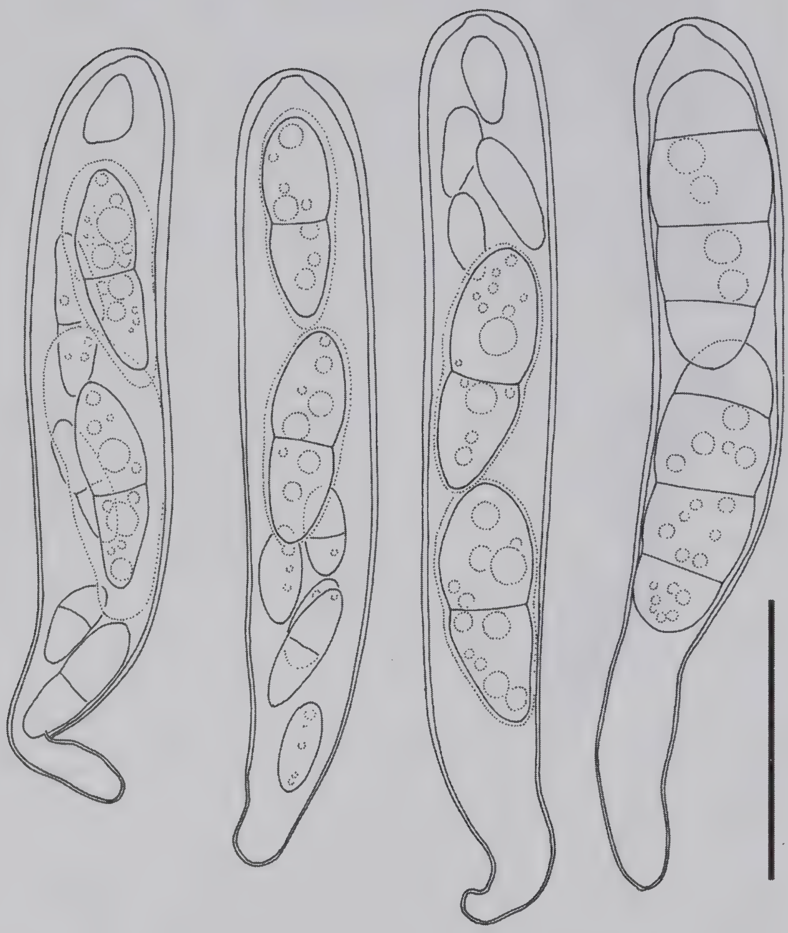


Fig. 2 – *Weddellomyces turcicus* (Holotype). Asci in water: three immature and one (far right) mature. Bar = 50 µm.

MYCOTAXON

Volume 94, pp. 253–263 October–December 2005

Cordyceps spegazzinii sp. nov., a new species of the *C. militaris* group

Mónica S. Torres[1], James F. White, Jr.[1] & Joseph F. Bischoff[2]

jwhite@aesop.rutgers.edu
[1]Department of Plant Biology and Pathology, Rutgers University
New Brunswick, New Jersey 08901

[2]National Center for Biotechnology Information, National Institutes of Health
Bethesda, Maryland 20894

Abstract—The proposed new species, *Cordyceps spegazzinii* sp nov., was collected on Barro Colorado Island, Panama. It is characterized by simple stromata with cylindrical to clavate heads, perithecia superficial to partially immersed, ascospores not fragmenting into part-spores and a *Evlachovaea* sp. anamorphic state. We compared *C. spegazzinii* to similar species in the *C. militaris* group. We determined that *C. spegazzinii* belongs to the *C. militaris* group, based on examination of morphological features and cultural characteristics, combined with phylogenetic analyses using LSU rDNA sequences.

Key words—*Clavicipitales*, taxonomy

Introduction

Members of the family *Clavicipitaceae* (*Hypocreales*) display a wide range of life strategies ranging from insect endoparasites to pathogens of grasses and endophytes (Spatafora et al. 1993). The entomogeneous fungal genus *Cordyceps* (Fr.) includes approximately 450 described species (Sung 2004, Stensrud et al. 2005) that are known to be endoparasites of several orders of arthropods and parasitic on hart's truffles (*Elaphomyces* spp.) (Kobayasi 1982, Mains 1957) while other species live on the sclerotia of plant pathogenic *Claviceps* species. In addition, a few species are known to utilize seeds of higher plants (Fukatsu & Nikoh 2003).

The genus *Cordyceps* has also received significant attention due to the importance of some species in the production of bioactive compounds. The species *C. sinensis* (Berk.) Sacc. and *C. sobolifera* (Hill) Berk. & Broome have been used in traditional Chinese medicine (Liu et al. 2002) and *C. kyusyuensis* Kawam. has recently received attention due to production of bioactive compounds with anti-tumor activity (Sun et al. 2003). Other species, like *C. brongniartii* Shimazu and *C. bassiana* Z.Z. Li et al. have been connected with their anamorphic stages, *Beauveria brongniartii* (Sacc.) Petch and *Beauveria bassiana* (Bals.-Criv.) Vuill., respectively, both with potential for biocontrol of insects (Shimazu et al. 1988, Li et al. 2001, Rehner et al. 2005).

There have been several attempts to better characterize the genus (Kobayasi 1982). Four subgenera have been proposed based on morphological characters: subgenera *Ophiocordyceps, Eucordyceps, Neocordyceps* (Kobayasi 1982) and *Bolacordyceps* (Erikkson 1986). However, identification and classification of *Cordyceps* species based exclusively on morphological and cultural data is a difficult task due to the large number of species that compose the genus, possible synonymies, presence of several anamorphs and inability to locate type specimens (Sung 2004, Sung & Spatafora 2004, Stensrud et al. 2005). Increasingly, phylogenetic studies have employed sequence data to shed light on the phylogenetic relationships and species delimitation in the genus *Cordyceps* (Sung et al. 2001; Nikoh & Fukatsu 2000, Sung et al. 2004, Stensrud et al. 2005). Sung et al. (2001) employed nuclear rDNA sequences (SSU and LSU) in the revision of *Verticillium* sect *Prostrata.* They determined that *Cordyceps* was not monophyletic and recognized two distinct clades, *C. militaris* sensu stricto and *C. ophioglossoides* clade. Stensrud et al. (2005) using ITS rDNA sequences of 72 clavicipitalean taxa also found *Cordyceps* to not be monophyletic and recognized four separate *Cordyceps* evolutionary lineages.

In 2003 a specimen of *Cordyceps* was collected on Barro Colorado Island, Panama. Morphological examination and phylogenetic placement of our material revealed that its features are not consistent with previously described *Cordyceps* species, and a new species is proposed.

Materials and Methods

Field collection: J.F. Bischoff and J.F. White in August 2003 made the collection on Barro Colorado Island, Panama. The material was associated with insect eggs on partially rolled leaves of an unknown dicotyledonous tree. The eggs were identified as belonging to Order *Diptera* by Karl Kjer (Department of Ecology and Evolution, Rutgers University). The material was brought to the field station and isolated on Potato Dextrose Agar (PDA; Difco, Inc.) with antibiotics (gentamicin 40 mg/L, streptomycin 40 mg/L; penicillin 20 mg/L). Stromata were kept in 90% alcohol. Specimens of the collections were submitted to the Rutgers University Plant Pathology Herbarium (RUTPP).

Morphological observations: Microscopic examinations of anamorphic characters were made from cultures maintained on PDA and potato carrot-agar (PCA), at room temperature (23˚C) for 10 days. Slide preparations were mounted in lactic acid-cotton blue or water. Twenty measurements were made for each morphological feature.

To observe the structure of perithecia, specimens were fixed in 95% ethanol, dehydrated in 100% ethanol and infiltrated with LR White® acrylic embedding medium for 24 hours. Specimens were oriented in capsules containing embedding medium and cured in an oven (60ºC) for 24 hours. Sections approximately 1 µm thick were made using glass knives and stained in aniline blue (0.1% aqueous) followed by toluidine blue (0.1% aqueous). Photographs were made using a Nikon Coolpix digital camera.

Cultural studies: Growth was measured on several media: PDA, PCA, malt-extract agar (MEA), Czapek cellulose agar (CCA) and corn meal dextrose peptone (CMDP). All media were inoculated using 7mm plugs cut from margins of colonies growing on PDA plates. Plates were maintained at 23°C in dark and measured after 5 days. Representative cultures were sent to the ATCC in Manassas, Virginia (MYA-3684) and USDA-ARS Collection of Entompathogenic Fungal Cultures (ARSEF) (ARSEF 7850).

Sequence data: DNA extraction was carried on from fresh perithecia taken in the field and from fresh mycelium growing on PDA media overlaid with cellulose acetate sheets. Genomic DNA was extracted using the DNeasy® Plant Mini Kit (Qiagen). Internal transcribed spacer (ITS) regions 1 and 2, 5.8s, and the 5' end of the large subunit LSU rDNA were amplified from 4 µL of genomic DNA using primers ITS5 and ITS4 (Sullivan et al. 2000) in a 50 µL reaction. PCR reactions, sequencing reactions to amplify the ITS and LSU regions, and reaction analyses were performed as described by Sullivan et al. (2000). The rDNA LSU and ITS1-5.8s-ITS2 regions were submitted to GenBank (accession number DQ196435).

Phylogenetic analysis: Sequencher (Genecodes, Ann Arbor, MI) was used to analyze, edit and construct consensus sequence from sequence products. Forty LSU sequences to represent *Cordyceps* species and others representatives of Clavicipitaceae were selected. GenBank accession numbers are listed in Table 1. Members of the family *Hypocreaceae* were used as outgroup taxa based on the work of Spatafora et al. (1993) and Nikoh et al. (2000). Sequences were aligned by hand using the secondary structure of *Saccharomyces cerevisiae* (U53879) from the comparative RNA Web site (CRW) database (Cannone et al. 2002). The matrix was annotated as described by Kjer (1995). Sequences were analyzed using the program PAUP v4.0 (Swofford 2002) by maximum likelihood and Bayesian analysis. ModelTest v.3.06 (Posada & Crandall 1998) was used to select the best fitting model of sequence evolution determined by Akaike information criterion (Akaike 1974). The model selected was GTR with proportion of invariable sites (I) and gamma distribution (G). The parameters include base frequencies A=0.2804, C=0.2443, G=0.373, T=0.1680; rate matrix [A-C]=0.4817, [A-G]=1.4368, [A-T]=1.021, [C-G]=1.0609, [C-T]=7.4863; [G-T]=1; I=0.6130 and G=0.5932 and this model was incorporated into PAUPv.4.0. The most likely tree (-ln 3703.8447) is shown in Fig. 11. Bayesian inference was used to estimate branch support (posterior probability) under likelihood using Mr Bayes 3.0 (Huelsenbeck 2000) (Fig. 11). Bayesian analysis was run three times with four mcmc (Markov Chain Monte Carlo) chains for 1,000,000 generations, sampling every 100 generations. The 30,000 trees resulting from the three runs were pooled and 28,500 were imported into PAUP to construct a majority rule consensus tree after discarding the asymptotic trees (burn in).

Five complete ITS sequences were selected based on being included in *C. militaris* clade or morphological similarities. GenBank accession numbers are listed in Table 1. Sequences were aligned using ClustalX (Thompson et al. 1997) and their similarity compared (Table 2).

Table 1. Taxa used in LSU and ITS analyses

Taxa	LSU	ITS
Atkinsonella hypoxylon	U57087	
Balansia henningsiana	U57678	
Balansia strangulans	U57679	
Beauveria brongniartii	AB027381	
Claviceps fusiformis	U17402	
Claviceps paspali	U47826	
Claviceps purpurea	U57085	
Cordyceps capitata	U57086	
Cordyceps cardinalis 1	AY184965	
Cordyceps cardinalis 2	AY184964	
Cordyceps gunnii	AF339522	AJ536552
Cordyceps inegoensis	AB027368	
Cordyceps japonica	AB027367	
Cordyceps kanzashiana	AB027371	
Cordyceps kyusyuensis	AY465959	AY781661
Cordyceps militaris	AB027379	AJ786573
Cordyceps ophioglossoides	U47827	
Cordyceps prolifica	AB027370	
Cordyceps pruinosa	AB044635	
Cordyceps pseudomilitaris	AF327376	AJ786589
Cordyceps ramosopulvinata	AB027372	
Cordyceps sinensis	AB067737	
Cordyceps spegazzinii	DQ196435	DQ196435
Cordyceps subsessilis	AF373285	
Cordyceps takaomontana	AB044637	AB044637
Cordyceps tuberculata	AF327384	
Dussiella tuberiformis	U57083	
Epichloe amarillans	U57680	
Epichloe typhina	U17396	
Hypocrea lutea	AB027384	
Hypocrea schweinitzii	U47833	
Hypomyces armeniacus	AF160293	
Hypomyces orthosporus	AF160241	
Lecanicillium lecanii 1	U17414	
Lecanicillium lecanii 2	U17421	
Neotyphodium coenophialum	AF160241	
Paecilomyces farinosus	DQ067297	
Paecilomyces tenuipes	AB027380	
Tolypocladium cylindrosporum	AF245301	
Tolypocladium inflatum	AB103381	

Taxonomic Description

Cordyceps spegazzinii M.S. Torres, J.F. White & J.F. Bischoff **sp. nov.**

FIGURES 1-4, 5-10

> *Stromata solitaria, simplicia in ovis insectorum (Diptera), 7-9 mm altus. Stipes tenuis, glabro. Zone fertilis terminalis, cylindricea vel clavata (1x2-3 mm). Perithecia agregata, laxe immersa, 400-460 x 200-260 µm ovata vel obclavata. Asci cylindracei, 200-250 x 2.5-3 µm, hyalini. Ascosporae hyalinie, filiformes, flexuosae, multiseptatae, 100-250 x 0.5-1 µm, in ascis numquam individuales in partes secedentes. Status anamorphicus in cultura PDA, celeriter crescens, mycelio aerio gossypino, hyphis vegetativis ramosis, septatis, albis, 1-2 µm latis, reverso cremeo. Cellulae conidiogenae monophialidicae, hyalinae, leves, 7-15 µm, ad septum 1-2. Conidia hyalina, aseptata, levia, ellipsoidea, 4-5 x 2 µm, catenulata* ***Etymology****: this species is named after the mycologist and naturalist Carlos L. Spegazzini.*
>
> ***Holotype***: Barro Colorado Island (BCI), Panama; insect eggs (Diptera) on leaves of unknown dicotyledonous plant; August 2003; J.F. Bischoff & J.F. White; Rutgers Mycological Herbarium (RUTPP).

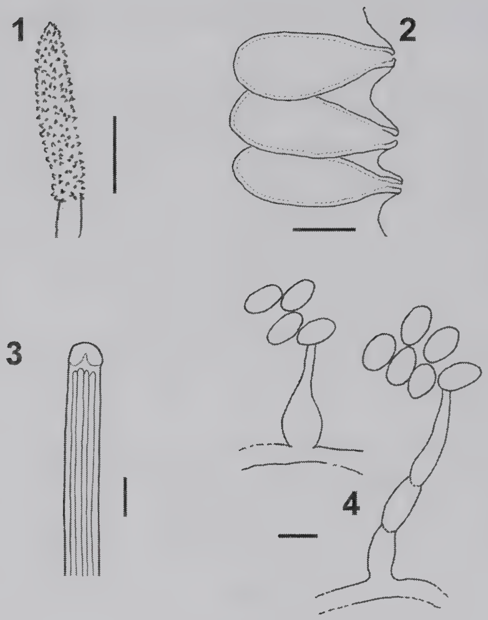
Stromata solitary, simple, associated to dipteran eggs, measuring 7-9 mm from base to tip, consisting of white, slender smooth stipe and yellowish, cylindrical to clavate head (measuring 1 x 3-4 mm) (Fig 1 and 5). Perithecia crowded, 400-460 x 200-240 µm, ovate to obclavate, superficial to partially immersed, with perithecial necks protruding; asci 200-250 x 2.5-3 µm (Fig. 6 and 7), cylindrical with a refractive apical cap (Fig. 3), containing 8 ascospores. Ascospores filiform, running in parallel, hyaline, irregularly multiseptate, 100-250 x 0.5-1 µm not fragmenting into partspores.

Colonies on PDA fast growing (20 mm in 5 days at 25°C; 14 mm in MEA, 15 mm in PCA, 18 mm in CMDP and 13 mm CCA), smooth surface, white, cottony, dense, with abundant sporulation; reverse cream to yellowish and smooth. *Evlachovaea* Borisov & Tarasov (Borisov & Tarasov 1999) anamorphic state developing in culture (Fig. 4, 8-10). Conidiogenous cells flask shaped (phialide), 7-15 µm long, swollen at the base, base 3 µm wide, and gradually narrowed to a distinct neck (Fig. 4). Phialides borne singly, grouped in pairs or in loose clusters (Fig. 8). Conidia hyaline, unicellular, smooth, oval to fusoid shaped, 4-5 x 2 µm, forming short dry chains with zipper-like morphology due to alternating oblique orientation of successive conidia when formed (Fig. 4 and 8).

Discussion

The features of *C. spegazzinii* do not match any previously described species of *Cordyceps*. *Cordyceps spegazzinii* is comparable to *C. takaomontana* Yakush. & Kumaz, *C. memorabilis* (Ces.) Sacc., *C. pseudomilitaris* Hywel-Jones & Sivichai and *C. cardinalis* G.H. Sung & Spatafora, all morphologicaly similar and closely related species in the *Cordyceps* s.s clade (Sung & Spatafora 2004).

Nevertheless, *C. spegazzinii* is easily separated by morphological and microscopic characteristics from *C. takaomontana* and *C. memorabilis*, species that are characterized by capitate stromata and superficial perithecia while *C. spegazzinii* is non-capitate with a much less distinct fertile head. Further, the species *C. takaomontana*, and *C. memorabilis* are parasites of *Coleoptera* while *C. spegazzini* was found associated to *Diptera* eggs.



Figs. 1-4. Morphological features of *C. spegazzinii* and its *Evlachovaea* sp. anamorph. 1. Stroma (bar=1mm). 2. Arrangement of perithecia in the stroma (bar=160µm). 3. Tip of ascus showing the cap (bar= 3µm). 4. Typical phialides and conidia of the anamorph from culture on PDA (bar= 3µm).

Cordyceps pseudomilitaris shares many morphological features with *C. spegazzinii* such as small stromata, superficial to partially immersed perithecia, smooth filiform ascospores that do not break into partspores. Although Hywel-Jones (1994) stated in his description that there exists a wide variation in size and shape in the specimens collected, *C. pseudomilitaris* presents slightly longer and wider asci and elongated ellipsoid to elongated ovoid perithecia while *C. spegazzinii* is characterized by ovate to obclavate perithecia and slightly shorter and thinner asci. Also *C. pseudomilitaris* was associated with a *Hirsutella*-like anamorph (Hywel-Jones 1994), and leptidopteran host. *Cordyceps cardinalis* differs from *C. spegazzinii* in that the fertile area of the former is reddish orange to reddish, cylindrical, elliptical to fusiform and those of the latter are yellowish cylindrical to clavate. Conidial state of *C. cardinalis* and *C. spegazzinii* are closely similar. The anamorph of *C. cardinalis* is characterized by phialides with swollen basal portions and producing conidia in partially imbricate chains and it was best described as being *Clonostachys*-like or *Mariannaea*-like (Sung & Spatafora 2004). *Cordyceps spegazzinii* anamorph present flask shaped phialide, with conidia forming short dry chains with zipper-like morphology. Humber et al. (2002) proposed

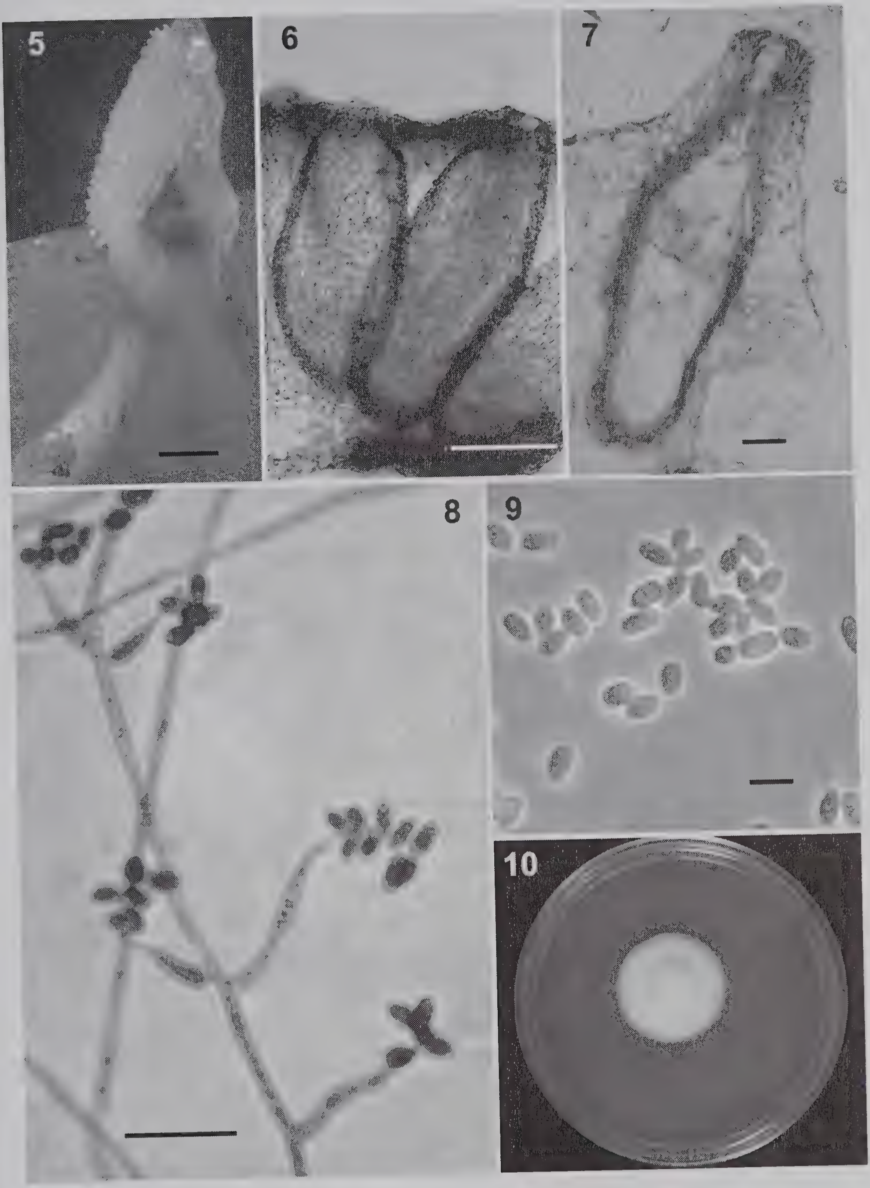


Fig. 5-10. *Cordyceps spegazzinii*. 5. Stroma (bar = 1 mm). 6. Cross section of stroma with perithecia. (bar = 150 µm). 7. Detail of perithecium (bar = 70µm). 8. Phialides (bar = 15µm). 9. Conidia (bar = 5 µm). 10. Seven-day-old colony on PDA.

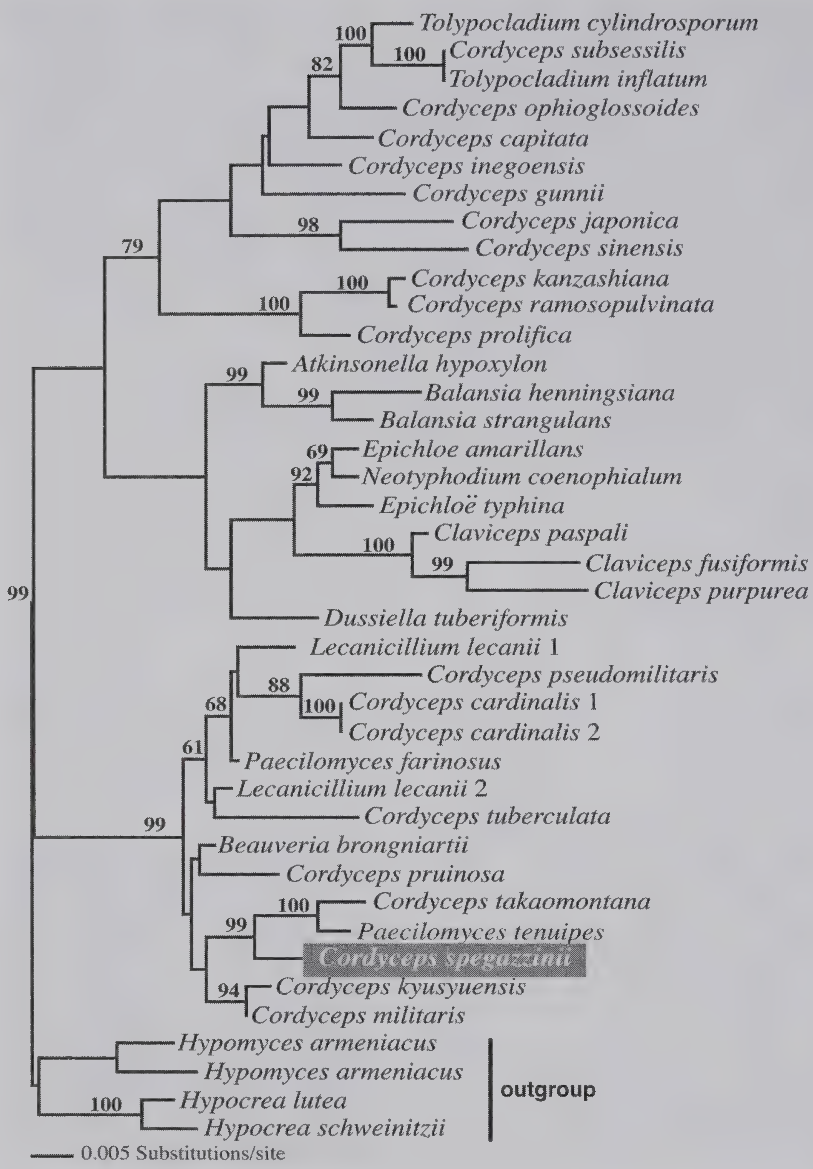

Tolypocladium cylindrosporum
Cordyceps subsessilis
Tolypocladium inflatum
Cordyceps ophioglossoides
Cordyceps capitata
Cordyceps inegoensis
Cordyceps gunnii
Cordyceps japonica
Cordyceps sinensis
Cordyceps kanzashiana
Cordyceps ramosopulvinata
Cordyceps prolifica
Atkinsonella hypoxylon
Balansia henningsiana
Balansia strangulans
Epichloe amarillans
Neotyphodium coenophialum
Epichloë typhina
Claviceps paspali
Claviceps fusiformis
Claviceps purpurea
Dussiella tuberiformis
Lecanicillium lecanii 1
Cordyceps pseudomilitaris
Cordyceps cardinalis 1
Cordyceps cardinalis 2
Paecilomyces farinosus
Lecanicillium lecanii 2
Cordyceps tuberculata
Beauveria brongniartii
Cordyceps pruinosa
Cordyceps takaomontana
Paecilomyces tenuipes
Cordyceps spegazzinii
Cordyceps kyusyuensis
Cordyceps militaris
Hypomyces armeniacus
Hypomyces armeniacus
Hypocrea lutea
Hypocrea schweinitzii
outgroup
100
100
82
98
79
100
100
99
99
69
92
100
99
99
88
100
68
61
99
100
99
94
100
0.005 Substitutions/site

Fig 11. The most likely tree based on rDNA LSU sequence data analysis using GTR+I+G model of evolution. The numbers on the branches indicate posterior probabilities

that morphological characteristics and entomopathogenic habit of *Evlachovaea* could suggest a teleomorph connection with *Cordyceps* species and certainly this is the case for *C. spegazzinii*. *Cordyceps cardinals* anamorph belongs to *Evlachovaea* genus (R. Humber, personal communication).

Host range of *Cordyceps* species may be of low significance as a phylogenetic character (Stensrud et al. 2005). Several *Cordyceps* species have been previously reported on hosts from Order *Diptera*, including *C. dipterigena* Berk. & Broome, *C. sakishimensis* Kobayasi & Shimizu, *C. discoideocapitata* Kobayasi & Shimizu, *C. bicephala* Berk., *C. iriomoteana* Kobayasi & Shimizu and *C. novoguineensis* Kobayasi & Shimizu. However, none of these species are grouped in subsection *Pseudoimmersae* (Kobayasi 1982) where *C. spegazzinii* would be placed based on its stromatic features.

Although the interrelationship of groups changed to some extent our analyses recognized the same general clades reported by Sung et al. 2001 and Sung & Spatafora 2004 (Fig.11). The *Cordyceps* s.s. clade (99% posterior probabilities) was identical to that observed by Sung & Spatafora (2004) and Stensrud et al. (2005) except for the addition of *C. spegazzinii*. Within this clade, *C. spegazzinii* grouped closely (99% posterior probabilities) with *C. takaomontana* and its anamorph *Paecilomyces tenuipes* (Peck) Samson (Luangsa-ard et al. 2005) and it is closely related to *C. kyusyuensis* Kawam. and *C. militaris* (L.) Fr. *Cordyceps spegazzinii* was compared to *C. kyusyuensis* and *C. militaris*. Morphologically, *C. spegazzinii* is distinguished from *C. kyusyuensis* in having ovate to obclavate superficial perithecia, and *Diptera* host while the later present ovoid immersed perithecia, *Verticillium*-like anamorphic state (Sung, personal communication) and leptidopteran host. *Cordyceps militaris* differs form *C. spegazzinii* in that the ascospores of the former disarticulate and those of the latter do not disarticulate. Additionally, *C. militaris* is characterized by a *Lecanicillium* W. Gams & Zare anamorph and brightly colored stromata.

In our analysis of ITS sequences similarities (Table 2), *C. takaomontana* was 93.2% and *C. gunnii* was only 78.3% similar to *C. spegazzinii.*

Table 2. Similarity matrix derived from the sequence data of the ITS1 region in six *Cordyceps* species.

Taxa	(1)	(2)	(3)	(4)	(5)	(6)
(1) *C. spegazziniii*	---					
(2) *C. takaomontana*	93.2	---				
(3) *C. militaris*	92.8	99.6	---			
(4) *C. kyusyuensis*	92.7	99.4	99.7	---		
(5) *C. pseudomilitaris*	90.2	94	94.2	93.5	---	
(6) *C. gunnii*	78.3	85	86.5	82.6	87.2	---

Acknowledgements

This research was supported in part by Fulbright Comission-Bunge & Born (Argentina, Grant 1512034) to M.S.T. We are grateful to Drs. Z.Q. Liang and R.A. Humber for review versions of the manuscript. We would like to acknowledge Dr. R.A. Humber for bringing the description of the genus *Evlachovaea* to our attention.

Literature cited

Akaike H. 1974. A new look at the statistical model identification. IEEE Transactions on Automatic Control 19:716-723.

Borisov BA, Tarasov KL. 1999. Notes on biodiversity of causal agents of invertebrate mycoses in Adjaria (southwestern Georgia). I. *Evlachovaea kintrischica* gen et sp. nov. (Hyphomycetes) from Kintrishi reservation. Mikol. Fitopatol. 33:248-256.

Cannone JJ, Subramanian S, Schnare MN, Collet JR, D'Souza LM, Du Y, Feng B, Lin N, Madabusi LY, Muller KM, Pande N, Shang Z, Yu N, Gutell RR. 2002. The comparative RNA Web (CRW) site: an online database of comparative sequence and structure information for ribosomal, intron, and other RNAs. BMC Bioinformatics 3:2.

Eriksson OE. 1986. Notes on ascomycete systematics. Systema Ascomycetum 5:113-174.

Huelsenbeck JP. 2000. MrBayes: Bayesian inference of phylogeny. Distributed by the author. Department of Biology, University of Rochester.

Humber RA, MR Tanzini, Alves SB. 2002. *Evlachovaea*: First reports of an unusual and little known entomopathogenic fungal genus from the new world. 35th Annual Meeting of the Society for Invertebrate Pathology, Fos do Iguassu, Brazil.

Hywel-Jones NL. 1994. *Cordyceps khaoyaiensis* and *C. pseudomilitaris*, two new pathogens of lepidopteran larvae from Thailand. Mycological Research 98:939-942.

Kjer KM. 1995. Use of rRNA secondary structure in phylogenetic studies to identify homologous positions: an example of alignment and data presentation from the frogs. Molecular Phylogenetics and Evolution 4:314–330.

Kobayasi Y. 1982. Keys to the taxa of the genera *Cordyceps* and *Torrubiella*. Trans. Mycol. Soc. Japan 23:329-364.

Li Z, Li C, Huang B, Fan M. 2001. Discovery and demonstration of the teleomorph of *Beauveria bassiana* (Bals.) Vuill. , an important entomogeneous fungus. Chinese Science Bulletin 46:751-753.

Liu Z, Liang Z, Liu A, Yao Y, Hyde K, Yu Z. 2002. Molecular evidence for teleomorph-anamorph connections in *Cordyceps* based on ITS-5.8S rDNA sequences. Mycological Research 106:1100-1108.

Luangsa-ard JJ, Hywel-Jones NL, Manoch L, Samson R. 2005. On the relationships of *Paecilomyces* sect. *Isarioidea* species. Mycological Research 109:581-589.

Mains EB. 1957. Species of *Cordyceps* parasitic on *Elaphomyces*. Bulletin of the Torrey Botanical Club 84:243-251.

Nikoh N, Fukatsu T. 2000. Interkingdom host jumping underground: phylogenetic analysis of entomoparasitic fungi of the genus *Cordyceps*. Molecular Biology and Evolution 17(4):629-638.

Fukatsu T, Nikoh N. 2003. Interkingdom host shift in the *Cordyceps* fungi. In: Clavicipitalean fungi: evolutionary biology, chemistry, biocontrol and cultural impacts. Marcell-Dekker, New York. Pp. 311-327.

Posada D, Crandall KA. 1998. Modeltest: testing the model of DNA substitution. Bioinformatics 14:817–818.

Rehner SA, Buckley E. 2005. A *Beauveria* phylogeny inferred from nuclear ITS and EF1-α sequences: evidence for cryptic diversification and links to *Cordyceps* teleomorphs. Mycologia 97:84-98.

Shimazu M, Mitsuhashi W, Hashimoto H. 1988. *Cordyceps brongniartii* sp. nov. the teleomorph of *Beauveria brongniartii*. Transactions of the Mycological Society of Japan 29:323-330

Spatafora JW, Blackwell M. 1993. Molecular systematics of unitunicate perithecial ascomycetes: The *Clavicipitales-Hypocreales* connection. Mycologia 85:912-922.

Stensrud O, Hywell-Jones N, Schumacher T. 2005. Towards a phylogenetic classification of *Cordyceps*. Mycological Research 100:41-56.

Sullivan RF, Bills GF, Hywel-Jones N, White JF Jr. 2000. *Hyperdermium* a new clavicipitalean genus for some tropical epibionts of dicotyledonous plants. Mycologia 92:908-918.

Sun Y, Ling L, Zhang J, Chen H, Zang C. 2003. Nucleoside from *Cordyceps kyushuensis* and the distribution of two active components in its different parts. Acta Pharmaceutica Sinica 38(9):690-694.

Sung GH, Spatafora JW, Zare R, Hodge KT, Gams W. 2001. A revision of *Verticillium* section Prostrata. II. Phylogenetic analyses of SSU and LSU nuclear rDNA sequences from anamorphs and teleomorphs of the Clavicipitaceae. Nova Hedwigia 72: 311-328.

Sung GH, Spatafora JW. 2004. *Cordyceps cardinalis* sp. nov., a new species of *Cordyceps* with a east Asian-eastern North American distribution. Mycologia 96: 658-666.

Sung J. 2004. *Cordyceps* diversity and its preservation in Korea. Supplement to Mycologia 55(4):1-3.

Swofford DL. 2002. PAUP*. Phylogenetic analysis Using Parsimony (*and Other Methods), Version 4. Sinauer Associates, Sunderland, Massachusetts.

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG. 1997. The ClustalX windows interface: flexible strategies for multiple alignment aided by quality analysis tools. Nucleic Acids Res 25:4876-4882.

MYCOTAXON

Volume 94, pp. 265–292 October–December 2005

A phylogeny of *Ramariopsis* and allied taxa

Ricardo García–Sandoval
Joaquín Cifuentes

garcia_s@ciencias.unam.mx
Herbario FCME, UNAM, Apdo. Post. 70-181
México DF., 04510, México

Efraín De Luna

Instituto de Ecología A.C., Apdo. Post. 63
Xalapa Veracruz, 91000, México

Arturo Estrada–Torres

CICB, Universidad Autónoma de Tlaxcala, Apdo. Post. 183
Tlaxcala, 90000, México

Margarita Villegas

Herbario FCME, UNAM, Apdo. Post. 70-181
México DF., 04510, México

Abstract—The phylogenetic relationships of *Ramariopsis* and related taxa were studied through a cladistic analysis of 36 morphological, cytological, and biochemical characters among 23 species in six genera. Two of these genera were directly studied as groups of interest, three as external taxonomic outgroups, and one as operative outgroup. Representatives of *Ramariopsis* sensu Corner formed a monophyletic group, supported by the cyanophilous nature of their basidiospores and derivation of their ornamentation from the tunica. The new combination, *Clavulinopsis antillarum,* is proposed.

Key words—*Clavariaceae*, Homobasidiomycetes, spore ultrastructure, outgroup sampling, taxonomy

Introduction

Ramariopsis was described as a subgenus of *Clavaria* by Donk (1933), who selected *Clavaria kunzei* Fr. as the type species; the name refers to its macromorphological similarity to the genus *Ramaria* (Donk 1954). The taxa originally included the type species, *Clavaria angulispora* Pat. & Gaillard, *Clavaria pulchella* Boud., *Clavaria pyxidata* Pers. and *C. subtilis* Pers.

Corner (1950) elevated the taxon to genus level and included several fibulate species with branched, whitish basidiomes, monomitic hyphal systems, and echinulate spores. He retained *Clavaria kunzei* [= *Ramariopsis kunzei* (Fr.) Corner] as the type species, removing three species—*C. angulispora*, *C. pyxidata* and *C. subtilis*—and adding eight more for a total of ten species in the genus. Petersen (1964) added two more species after examining the type specimens of various species of the genus *Clavulinopsis*.

Petersen (1966) emended the original delimitation of the genus, to include taxa with smooth spores and hysterochroic basidiomes. Petersen considered the size of the basidia, the thickness of the spore wall, the composition of the ornamentation—when present—and the pattern of coloration of the basidiomes as the relevant characters for circumscribing the genus. He proposed dividing *Ramariopsis* into two subgenera: *Laevispora,* typified by *Ramariopsis minutula* (Bourdot & Galzin) R.H. Petersen, for species with smooth-spores, and *Ramariopsis*, consisting of species with echinulate spores.

Corner (1970) maintained the original circumscription of the genus, recognizing that it might be an artificial group, closely related to *Scytinopogon* and *Clavulinopsis*. Corner argued that his circumscription conformed to a homogeneous group that was of more utility for fieldwork.

Petersen (1978a) proposed a new delimitation for the genera *Ramariopsis*, *Clavulinopsis*, and *Clavaria* based on the size of the hilar appendix, the type of pigments present in the basidiome and the number of nuclei remaining in the basidium after the formation of spores. He transferred species with globose spores and a conspicuous hilar appendix from *Clavulinopsis* to *Ramariopsis*, and species with elongate spores and a small hilar appendix from *Clavulinopsis* to a new subgenus: *Clavaria* subg. *Clavulinopsis*. He also proposed designating *Clavaria corniculata* Schaeff. [= *Ramariopsis corniculata* (Schaeff.) R.H. Petersen] as the type species of *Ramariopsis*.

Based on Petersen's arguments, *Ramariopsis* should include hysterochroic species with branched or simples basidiomes, with whitish or bright coloration, globose or subglobose and smooth or ornamented spores, and a conspicuous hilar appendix (see Fig. 1). Petersen argued that this circumscription permits a continuum among related species, from smooth-spored species with a large basidiome, to species with a small basidiome and echinulate spores.

There are only a few additional contributions to this polemic. Jülich (1985) transferred all species of *Ramariopsis* to *Clavulinopsis* based on nomenclatural arguments, but this interpretation apparently has not been followed by the majority of taxonomists (Hawksworth et al. 1995, Kirk et al. 2001). Pegler & Young (1985), in an electron microscopy (EM) study of several species of *Ramariopsis*, *Clavulinopsis*, and *Scytinopogon*, described three ultrastructural patterns of spore ornamentation that corresponded with the three genera mentioned before. They also observed that several apparently smooth-spored species, such as *Ramariopsis californica* R.H. Petersen, actually possessed ornamentation. The observed ornamentation was very small and covered by a thin myxosporium, such that the spores appear smooth under a light microscope even at magnifications above 1000x. Pegler & Young (1985) recognized

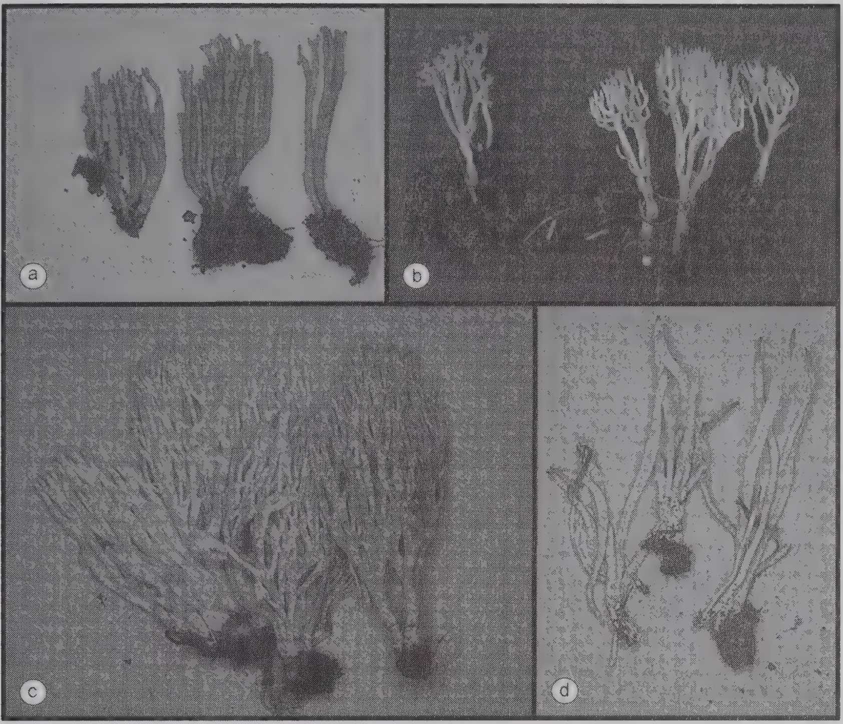


Figure 1. Four species representative of the variation observed in *Ramariopsis* and *Clavulinopsis*. a) *Clavulinopsis corniculata*, b) *Ramariopsis pulchella*, c) *Ramariopsis kunzei* and d) *Clavulinopsis fusiformis*. Photos a: J. Cifuentes, b: A. Estrada-Torres, c: J. Cifuentes, d: J. Cifuentes.

the delimitation proposed by Corner (1950) for *Ramariopsis*, but not the relationship between this genus and *Scytinopogon*.

The genus *Ramariopsis* has been included in the *Clavariaceae* in the majority of the treatments of this family (Donk 1964, Corner 1970, Jülich 1981, Hawksworth et al. 1995), with the exception of Petersen (1978a, 1988a) and Kirk et al. (2001), who placed *Ramariopsis* in *Gomphaceae*.

A phylogenetic study of *Gomphaceae* (Villegas et al. 1999), however, indicates that *Ramariopsis*—at least sensu Corner—should not be considered part of that family. Pine et al. (1999), on the other hand, studied the clavarioid and cantharelloid Homobasidiomycetes, and found that *Clavulinopsis fusiformis* (Sowerby) Corner [= *Ramariopsis fusiformis* (Sowerby) R.H. Petersen] nested within the euagaric clade, forming a monophyletic group with *Clavaria acuta* Sowerby, indicating that *Ramariopsis* subgenus *Laevispora* is related to *Clavaria*, or at least to representatives of *Clavaria* subgenus *Holocoryne*. Additionally results of Larsson et al. (2004) indicate close relationships among *Clavulinopsis helvola* (Pers.) Corner, *Clavaria argillacea* Pers.–*Clavaria* subgenus *Holocoryne*–and *Clavaria fumosa* Pers.–subgenus *Clavaria*–

on a monophyletic group nested in the euagaric clade. This results points to a close relationship between *Clavulinopsis* and at least some part of *Clavaria.*

There are currently only a few works, that have attempted to study the phylogeny of clavarioid and gomphoid macromycetes in general (Pine et al. 1999, Villegas et al. 1999, Humpert et al. 2001), and there is no consensus about the phylogenetic relationships of these taxa.

Given that there is no consensus delimitation for *Ramariopsis,* the number of species in this genus depends on the source consulted (i.e. Jülich 1981, 1985; Hawksworth et al. 1995; Kirk et al. 2001). This number varies from 24 to 45 species, with a distribution that stretches from sub-Arctic regions to the forests of New Zealand (Corner 1950, 1967a, 1970; Thin 1961; Petersen 1968, 1969, 1971a, 1978b, 1979, 1988a, 1989; Pilát 1971; Gómez 1972; García-Sandoval et al. 2002).

The principal objective of the present work is to suggest a more robust delimitation of *Ramariopsis,* based on a phylogenetic analysis of the available information and new morphological characters derived from direct observation of herbarium specimens. The use of morphological characters presents some advantages (see Jenner 2004, Wiens 2004, for an extensive up-to-date review) and for this particular case, these include the possibility of a wide sampling of species because of the availability of herbarium material and the opportunity to directly test the hypothesis of homology for diverse characters considered taxonomically relevant.

Materials and Methods

Selection of outgroups and taxonomic sampling. The selection of outgroups was critical for the present study since a reference phylogenetic framework is lacking and an inadequate or insufficient selection of external groups could result in the artificial interpretation of monophyly of the group of interest (Nixon & Carpenter 1993, Hopple & Vilgalys 1999). Selection was based on three criteria: a) a phylogenetic survey of the family *Clavariaceae* sensu lato (results not shown), b) previous phylogenies of the clavarioid Homobasidiomycetes, and c) previous classification proposals that include the genus *Ramariopsis* in some specific family.

The phylogenetic survey was conducted based on diverse delimitations of the family *Clavariaceae* (Donk 1964, Corner 1970, Jülich 1981, Hawksworth et al. 1995). All of the genera included in these proposals were considered, and representatives of the observed variation were selected for study. A matrix of 26 taxa and 30 morphological characters was constructed, and an initial selection of taxonomic outgroups and an operative outgroup was performed based on the strict consensus of the trees obtained from the analysis. The selection of outgroups, especially the operative outgroup, was based partially on the phylogenetic analyses by Hibbett et al. (1997), Pine et al. (1999), Humpert et al. (2001) and Binder & Hibbett (2002). Based on Petersen's (1978a, 1988a) proposals of the phylogenetic affinities of *Ramariopsis*, one additional representative of *Gomphus* was selected to complete the taxonomic outgroups. Sampling of the ingroup was based on Petersen's (1978a) proposed delimitation of *Ramariopsis*, which includes the species considered by Corner (1950). Taxa representative of the observed variation, and with available herbarium specimens, were chosen for analysis.

For a few confusing species [*Clavaria sulcata* (Overeem) R.H. Petersen, *Clavaria vermicularis* Sw., *Clavaria amoena* Zoll. & Moritzi and *Clavaria aurantiocinnabarina* Schwein.], assignment of specific epithets and the concepts used to delimit species followed Petersen (1967, 1976, 1979, 1980a, 1988a).

Analysis of characters and elaboration of the data matrix. Morphological observations were analyzed and interpreted in the framework of cladistic ontology (Hennig 1966, Farris 1983, de Pinna 1991, De Luna & Mishler 1996). The selection and analysis of characters were based on the variation observed among sampled species, without excluding a priori any sources of information (Poe & Wiens 2000). Hypotheses of homology were elaborated based on the homology criteria proposed by de Pinna (1991), employing similarity, conjunction, independence, variability, and heritability as auxiliary criteria (Patterson 1988, Rieppel 1988, Brower & Schawaroch 1996, Hawkins et al. 1997, Rieppel & Kearney 2002).

Codification of characters followed the criteria proposed by de Pinna (1991) and later additions (Hawkins et al. 1997, Hawkins 2000, Kluge 2003, Grant & Kluge 2004). Characters were not ordered nor polarized a priori to avoid bias in the exploration of tree space (Hauser & Presch 1991). Similarly, no weighting scheme was applied a priori to avoid ad hoc hypotheses that would constrain the results (Farris 1983). Character states were analyzed by directly observing herbarium specimens from distinct collections (see Table 1); these data were complemented by previous descriptions (Coker 1923; Singer 1945, 1986; Corner 1950, 1957, 1966, 1967a, b, 1970; Thin 1961; Petersen 1964, 1965, 1966, 1967a, 1968, 1969, 1971a, b, 1978b, c, d, 1979, 1980b, 1984, 1985, 1988a, b, 1989; Petersen & Olexia 1967, 1969; Bataile 1969; Fiasson et al. 1970; Schild 1971; Kühner 1977; Hubbard & Petersen 1979; Claus 1983; Pegler & Young 1985; Gill & Steglich 1987; Hansen & Knudsen 1997; García-Sandoval et al. 2002; Gill 2003; Bertagnolli & Novello 2004). A matrix of 36 characters (see Appendices 1 and 2) was constructed, that included observations of macro- and micro-morphology, macro– and micro–chemical reactions, and biochemical, cytological, and ultrastructural characters. Information for the homology hypothesis came primarily from direct observation of herbarium specimens and only in few cases were based on previously reported data (see Appendices).

Tree searches, robustness, and topology test. A series of heuristic searches were performed with 1,000 replicates in PAUP* 4.0b10 (Swofford 2002), using TBR, random addition, and MAXTREE set to auto-increase. A branch and bound search was performed using as an upper limit the observed tree length from the heuristic searches, and characters were optimized with the ACCTRAN option.

Interpretation of the change of character states along phylogenies was made in WinClada (Nixon 2002), using one of the most parsimonious trees encountered in the branch and bound search.

Bremer's support (Bremer 1994) was calculated to evaluate the robustness of the observed clades. The analysis was conducted using AutoDecay 4.0 (Eriksson 1999) with 100 heuristic replicates per search, using random addition, MAXTREE set to auto-increase, the ACCTRAN option for optimization, and equally weighted characters. Bootstrap values (Felsenstein 1985) were also calculated using 10,000 replicates sampling all characters, with 10 heuristic searches for each bootstrap replicate, TBR branch rearrangement, and MAXTREE set to 100 trees.

Table 1. List of specimens examined.

Species		Specimens
Clavaria amoena	TO	Corner CLAVARIA–4 (E); Donk 13690 (L)
Clavaria aurantiocinnabarina	TO	Cifuentes 2004–94 (FCME); Corner RSNB–8376 (L); Corner RSNB–8378A (L); Corner ICTA–1501 (E)
Clavaria gibbsiae Ramsb.	TO	Corner 442 (L); Corner 24165 (L); Corner–Singer 24165 (E)
Clavaria sulcata	TO	Hongo 705 (L); Corner s.n. (E); Corner 1676 (E)
Clavaria vermicularis	TO	Brit. Mycol. Soc. 12099 (L); Kotlaba s.n. (L); Corner NG 192 (E); Corner RSS 1439 (E)
Clavaria zollingeri Lév.	TO	Corner s.n. (E); Corner s.n. (E)
Clavariadelphus pistillaris (L.) Donk	TO	Meyer 3700 (TENN); Petersen 4920 (TENN)
Clavulinopsis corniculata (Schaeff.) Corner	IG	Piepenbroek & Piepenbroek 876 (L); Mass Geesteranus 14580 (L); Villegas 1144 (FCME); López 782 (ENCB); Aranda-Breceda 4 (FCME); Corner & Thind 206 (E)
Clavulinopsis fusiformis	IG	Guzmán U-482 (XAL); Cooke & Cooke 45644 (XAL); Cooke & Cooke 39815 (XAL); Hongo 764 (L); Villegas 1313 (FCME); Villegas 1305 (FCME); Heredia 371 (XAL); Heredia 371 (XAL); Santillán s.n (XAL); Guzmán & Ventura 5835 (ENCB); Ventura 13281 (ENCB); Villegas 1438 (FCME)
Clavulinopsis helvola	IG	Bas 6730 (L); Maas Geesteranus 13887 (L)
Clavulinopsis laeticolor (Berk. & M.A. Curtis) R.H. Petersen	IG	Corner 452 (L); Donk 13896 (L); Villegas 1803 (FCME); Hernández 188 (IBUG); Altamirano 628 (TLXM); Villegas 1450 (FCME).
Gomphus clavatus (Pers.) Gray	OO	Petersen 1797 (TENN); Arias-Montes s.n (FCME)
Gomphus floccosus (Schwein.) Singer	TO	Cifuentes 111 (FCME); Moreno-Fuentes 418 (FCME); Villegas 1109 (FCME); Fajardo s.n (FCME).
Lactarius indigo (Schwein.) Fr.	TO	Mendoza 9–09–1983 (FCME)
Ramariopsis californica	IG	Petersen 3006 (TENN); Petersen 280109 (TENN)
Ramariopsis crocea (Pers.) Corner	IG	Loerakker s.n. (L); Jalink & Nauta 6384 (L); de Vries s.n. (L)
Ramariopsis kunzei	IG	Bas 5105 (L); Corner RSNB-8291; Petersen 3909 (TENN); Guzmán U-399 (ENCB); Petersen s.n. (TENN); Villegas 1804 (FCME); Pérez-Ramírez 280 (FCME); Rodríguez s.n. (ENCB); Guzmán-Dávalos 2848 (IBUG); Guzmán 22666 (ENCB); Guzmán 6969 (ENCB); Valenzuela 1197 (ENCB); Corner NG-237 (E); Corner NG-229 (E); Ruíz & Herrera 3494 (MEXU)
Ramariopsis pulchella (Boud.) Corner	IG	Corner NG-217 (E); Altamirano 148 (TLXM); Altamirano 157 (TLXM)
Ramariopsis tenuiramosa Corner	IG	Donk 11421 (L); Mass Geesteranus 9576 (L); Geesink 1504 (L); Corner NG-124 (E)
Scytinopogon dealbatus (Berk.) Corner	TO	Corner s.n. (E)
Scytinopogon echinosporus (Berk. & Broome) Corner	TO	Corner 1517 (E)
Scytinopogon robustus (Rick) Corner	TO	Cifuentes 676 (FCME); Cifuentes 2004–26 (FCME)
Scytinopogon pallescens (Bres.) Singer	TO	Martínez-C. s.n (ENCB)

Herbaria: E = Royal Botanic Garden, Edinburgh, Scotland, United Kingdom; ENCB = Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, México; FCME = Facultad de Ciencias, UNAM, México; IBUG = Instituto de Botánica, Universidad de Guadalajara, México; L = National Herbarium Nederland, Leiden University Branch, Nederlands; MEXU = Instituto de Biología, UNAM, México; TENN = University of Tennessee, Knoxville, EUA; TLXM = Centro de Investigación en Ciencias Biológicas, Universidad Autónoma de Tlaxcala, México; XAL = Instituto de Ecología, A.C., Xalapa, México; IG = Ingroup (= group of interest); TO = Taxonomical outgroup; OO = Operative outroup.

These parameters were selected to allow for a large number of bootstrap replicates and a reasonably accurate search procedure for each replicate (as opposed to the "fast bootstrap" option), thus avoiding the underestimation of clade support (DeBry & Olmstead 2000; Mort et al. 2000).

Templeton's topology test (Templeton 1983) was used to evaluate differences between the observed phylogenetic hypothesis and that of Petersen (1978a), employing a two-tailed Wilcoxon's signed rank test following Templeton (1983). To conduct the test, a branch and bound search was performed constraining monophyly of the representatives of *Ramariopsis* sensu Petersen. To select a subgroup of equally parsimonious trees for topology testing, a second branch and bound search was conducted using ACCTRAN to optimize characters and successive weighting (Farris 1969) following Carpenter (1988, 1994), using the RI to calculate reweighting. Each of the most parsimonious trees thus encountered was compared with each of the most parsimonious trees from the branch and bound search with successive weights (see above) using the Templeton test implemented in PAUP*b10 (Swofford 2002), and the results were compared to tables of critical values of T for the Wilcoxon test.

Diverse methods exist to evaluate the stability of a phylogeny with respect to the inclusion/exclusion of taxa (see Grant & Kluge 2003 for an extensive review). The present study assessed the impact of taxonomic outgroups sampling with a selective inclusion/exclusion of those taxa, followed with branch and bound searches of all of the combinations of taxonomic outgroups: *Clavaria, Clavariadelphus, Gomphus, Lactarius,* and *Scytinopogon.*

Results

Twenty–three species were chosen for analysis based on the criteria employed for outgroup selection, taxonomic sampling, and character analysis (Table 1). The branch and bound search resulted in 12 trees of 80 steps in length (CI = 0.4875, RI = 0.7153, RC = 0.3487). The strict consensus of these (see Fig. 2) shows *Ramariopsis* sensu Corner (1950, 1970) as a monophyletic group.

This group forms a monophyletic clade with the representatives of *Scytinopogon.* Bootstrap analysis indicated a generally low level of support across the observed clades (see Fig. 2); only the clades containing representatives of *Ramariopsis* Corner, and *Scytinopogon* showed bootstrap support above 50%. Interestingly, there was no significant support for the clade that includes most of the representatives of *Clavaria* sensu Petersen. Bremer support was also relatively low for most of the clades, and the highest values corresponded to the clade of *Scytinopogon.*

During the constrained analysis conducted for topology tests, 1540 equally parsimonious trees of length 85 (CI = 0.4535, RI = 0.6781, RC = 0.3075) were found, five steps longer than those in the unconstrained search.

The application of successive weightings allowed selection of a subset of 8 most parsimonious trees from those found in the original branch and bound search. Application of the topology test, however, did not indicate significant differences between the hypotheses. Comparison of all topologies resulted in no significant values of N = 16-14, T = 53-39.5, P = 0.4545-0.4220.

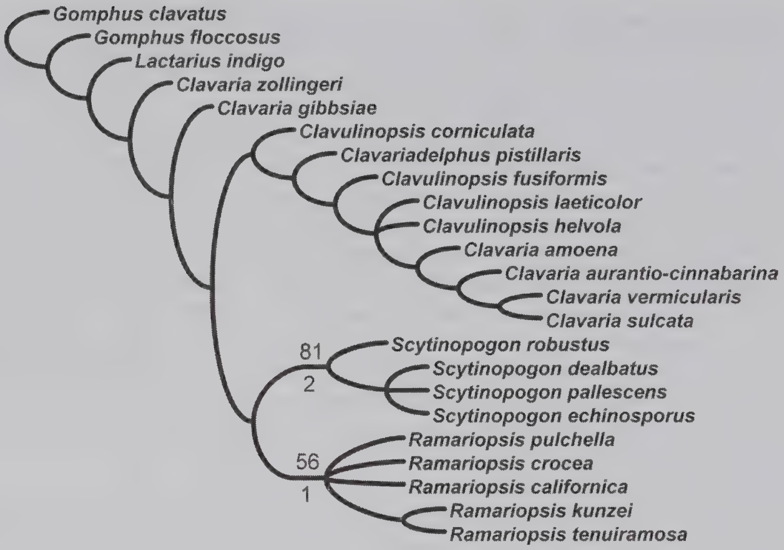

Gomphus clavatus
Gomphus floccosus
Lactarius indigo
Clavaria zollingeri
Clavaria gibbsiae
Clavulinopsis corniculata
Clavariadelphus pistillaris
Clavulinopsis fusiformis
Clavulinopsis laeticolor
Clavulinopsis helvola
Clavaria amoena
Clavaria aurantio-cinnabarina
Clavaria vermicularis
Clavaria sulcata
81
2
Scytinopogon robustus
Scytinopogon dealbatus
Scytinopogon pallescens
Scytinopogon echinosporus
56
1
Ramariopsis pulchella
Ramariopsis crocea
Ramariopsis californica
Ramariopsis kunzei
Ramariopsis tenuiramosa

Figure 2. Strict consensus of the 12 trees of 80 steps (CI = 0.4875, RI = 0.7153, RC = 0.3487) found during the branch and bound search. Numbers below branches indicate Bremer support indices, and numbers above branches indicate bootstrap support values.

The combinations of selective inclusion/exclusion tested (not shown) did not modify the monophyly of *Ramariopsis* sensu Corner, but decreased the resolution of the topology.

Discussion

The phylogenetic analyses performed support the monophyly of of *Ramariopsis* sensu Corner (1950), though with moderate bootstrap support (56%). Based on these results, *Ramariopsis* is limited to species with branched basidiomes, echinulate spores, and cyanophilous spore ornamentation derived from the tunica (Pegler & Young 1985).

The ultrastructural composition of the ornamentation is a synapomorphy for the group (see Fig. 3), but a large part of the cladogram was optimized as ambiguous for this character because of the lack of information for several species –e.g. information for any of the species of *Scytinopogon* included is not available. Although Corner did not include *Ramariopsis californica* in his most recent treatment of the genus (Corner 1970), this species exhibits all of the distinctive characters of the genus and the present results support its inclusion in this taxon. On the other hand, Petersen (1978a) included *Clavulinopsis helvola* in *Ramariopsis* [= *Ramariopsis helvola* (Pers.) R.H. Petersen], although this species has simple basidiomes and spores with thick tuberculous ornamentation. Pegler & Young (1985) established that this ornamentation is formed from growth of the corium, whereas ornamentation in *Ramariopsis* is formed via growth of the tunica. The results of the present study support the segregation of *C. helvola* from *Ramariopsis.*

The representatives of *Scytinopogon* form a well-supported group (81% bootstrap support), consistent with the original delimitation of Singer (1945) based on the presence of branched, thelephoroid basidiomes and verrucose spore ornamentation. In our results, *Ramariopsis* sensu Corner forms a monophyletic group with the representatives of *Scytinopogon*. These taxa all have cyanophilous spores with ornamentation partially derived from the tunica, though in *Scytinopogon* the ornamentation also seems to be composed of a thick core of corium (Pegler & Young 1985). It is worth mentioning that most of the species of *Scytinopogon* included in the present analysis have not been studied ultrastructurally—such data exist for only a single species of the genus, whose taxonomic status is in doubt.

The observed relationship between *Ramariopsis* and *Scytinopogon* was first suggested by Corner (1970), but this link should only be considered tentative as the taxonomic sampling of this analysis was designed to resolve a robust delimitation of the genus *Ramariopsis*, and not to identify its sister taxon. Furthermore, the clade *Ramariopsis+Scytinopogon* does not show bootstrap support. Thus, without a broader taxonomic sampling designed to establish the affinities of *Ramariopsis* with other taxa, it is preferable to consider these results as preliminary.

One of the principal consequences of this study is that *Ramariopsis* sensu Petersen is a paraphyletic group –i.e. a grade– because it was defined by simplesiomorphic characters. Petersen (1978a) used diverse sources of information for his delimitation, including the absence of carotenoid pigments. Pigment composition has been a frequently-used auxiliary character in fungal systematics (Arpin & Fiasson 1971, Tyler 1971, Gill & Steglich 1987, Frisvad et al. 1998, Gill 2003), but recent studies indicate that phylogenetic patterns inferred in the Homobasidiomycetes based on this type of character are often incongruent with the results obtained using other sources of information (Hibbett & Thorn 2001, Pine et al. 1999).

Nonetheless, the presence of certain types of pigments can be a very useful auxiliary character in studies aimed at generic delimitation (e.g. Feibelman et al. 1997, Weinstein et al. 2002), and the taxonomic relevance of this type of characters should not be completely discarded, but perhaps restricted to use at lower taxonomic levels. In the case of *Ramariopsis*, the delimitation proposed by Petersen (1978a) was based on the absence of carotenoid compounds, without specify the nature of the pigments present and without an explicit reference to a concrete character; the inferred pattern thus cannot be directly confirmed or refuted. In the present study this character was coded as the presence or absence of carotenoid pigments–character 11. (See Appendices for character argumentation.)

This character does not show evidence of homoplasy in the present study based on its observed distribution (CI = 1.0), although optimization of the character is not definitive due to the absence of information in several of the considered species (see Fig. 4). Additionally, the distribution of carotenoids in the present study supports the relationship among *Clavaria aurantiocinnabarina*, *C. amoena* and *C. sulcata*, and is congruent with a monophyletic group that includes representatives of *Clavaria* (see Fig. 2). This latter group is consistent with Petersen's (1978a, 1988a) delimitation of *Clavaria*. It is worth mentioning that the aforementioned results regarding *Clavaria* should be

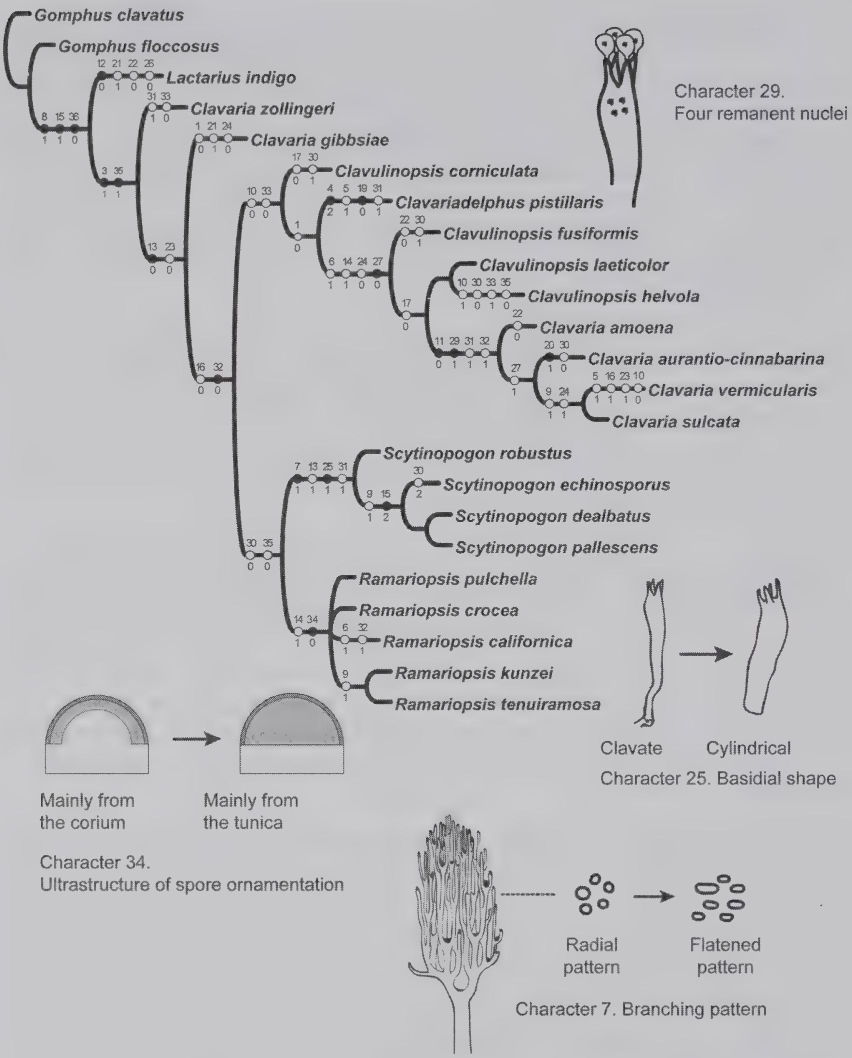

Gomphus clavatus
Gomphus floccosus
Lactarius indigo
Clavaria zollingeri
Clavaria gibbsiae
Clavulinopsis corniculata
Clavariadelphus pistillaris
Clavulinopsis fusiformis
Clavulinopsis laeticolor
Clavulinopsis helvola
Clavaria amoena
Clavaria aurantio-cinnabarina
Clavaria vermicularis
Clavaria sulcata
Scytinopogon robustus
Scytinopogon echinosporus
Scytinopogon dealbatus
Scytinopogon pallescens
Ramariopsis pulchella
Ramariopsis crocea
Ramariopsis californica
Ramariopsis kunzei
Ramariopsis tenuiramosa
Character 29.
Four remanent nuclei
Clavate
Cylindrical
Character 25. Basidial shape
Mainly from the corium
Mainly from the tunica
Character 34.
Ultrastructure of spore ornamentation
Radial pattern
Flatened pattern
Character 7. Branching pattern

Figure 3. One of the most parsimonious trees encountered during the branch and bound search, showing the character states that can be unambiguously optimized. Numbers above dots indicate the character and numbers below dots the character state. Apomorphic states are shown in black dots and homoplastic states in white dots. Selected character transformations are illustrated close to the branch were change occur (see appendix 1 for character argumentation).

considered as preliminary due to the lack of bootstrap support and the taxonomic sampling of the present study.

Another relevant character used by Petersen (1978a) was the presence of spores with a conspicuous hilar appendix. Several species of *Clavulinopsis* subgenus *Cornicularia* was transferred by Petersen to *Ramariopsis* based—in addition to the other mentioned character—on the presence of a conspicuous hilar appendix. In this study, this character was included (character 31) and their optimization is depicted in figure 4 (see also Appendices for character argumentation). The present results show it as a simplesiomorphy.

Farris (1991) provided an explicit criterion to recognize paraphyletic groups by tracing the status of the character used to define it; in the case of *Ramariopsis* prominent hilar appendix and absence of carotenoid pigments were used by Petersen in order to define the genus, both characters are plesiomorphic and shared –simplesiomorphic– (see Fig. 4). In the strict sense, the present results show *Ramariopsis* sensu Petersen as a paraphyletic group.

Petersen (1978a) also employed the presence of chiastic basidia with a post-meiotic mitotic division and four nuclei remaining in the basidia after spore formation as a cytological pattern that supported the delimitation of *Ramariopsis*. Recent phylogenetic studies (Hibbett et al. 1997, Pine et al. 1999) confirm the utility of cytological characters in the delimitation of taxonomic groups among the clavarioid and cantharelloid Homobasidiomycetes, but while the stictic pattern appears phylogenetically informative, the chiastic condition, which is widely distributed among the Homobasidiomycetes, does not seem to follow a clear phylogenetic pattern (Hibbett & Thorn 2001). As with the presence of carotenoid pigments, the utility of the chiastic condition in our analyses is noted, though more studies are necessary.

The pattern of four remaining nuclei reported for *Ramariopsis crocea* (Penancier 1961) results from a post-meiotic mitotic division. Post-meiotic mitotic divisions resulting in four nuclei remaining in basidia following spore formation are reported for a diversity of other taxa (Penancier 1961, Duncan & Galbraith 1972, Restivo & Petersen 1976, Kühner 1977, Mueller & Ammirati 1993). Both the meiotic pattern (chiastic/stictic) and the number of remaining nuclei (see Appendices for discussion and codification) were included as characters in the present study.

All of the species in our study for which data were available present a chiastic pattern, so it was not informative for addressing our questions. Very possibly this character could have relevance at other hierarchical levels when studying the taxonomic affinities of the genus *Ramariopsis*.

The number of remaining nuclei (character 29 CI = 1.0) did not present a homoplasious distribution, though, similar to the situation for carotenoid pigments, optimization of this character should be considered preliminary since data were not available for all species considered, and this lack of information results in a severely ambiguous optimization (results no shown).

Our finding of a monophyletic group that includes representatives of *Clavulinopsis* and representatives of *Clavaria* is congruent with the results of Pine et al. (1999). However, relationships among *Clavariadelphus pistillaris*, *Clavaria zollingeri* and *C. gibbsiae* are not consistent with previous classifications (see Fig. 2), and *Clavariadelphus* is found in a position incongruent with previous studies (Hibbett et al. 1997, Pine et al. 1999).

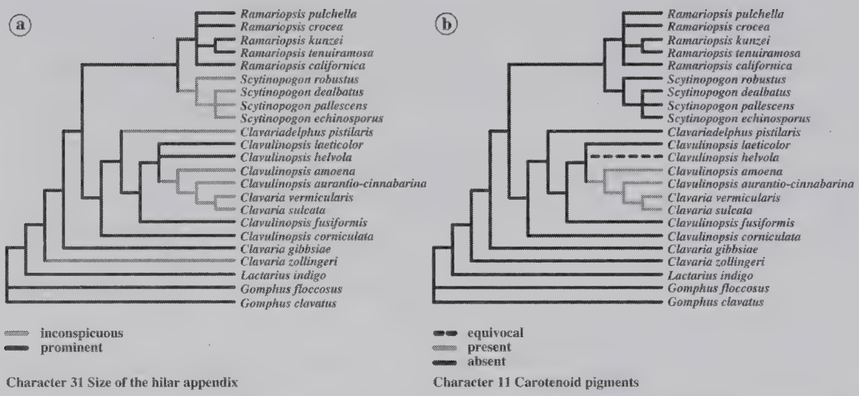

a
Ramariopsis pulchella
Ramariopsis crocea
Ramariopsis kunzei
Ramariopsis tenuiramosa
Ramariopsis californica
Scytinopogon robustus
Scytinopogon dealbatus
Scytinopogon pallescens
Scytinopogon echinosporus
Clavariadelphus pistilaris
Clavulinopsis laeticolor
Clavulinopsis helvola
Clavulinopsis amoena
Clavulinopsis aurantio-cinnabarina
Clavaria vermicularis
Clavaria sulcata
Clavulinopsis fusiformis
Clavulinopsis corniculata
Clavaria gibbsiae
Clavaria zollingeri
Lactarius indigo
Gomphus floccosus
Gomphus clavatus
inconspicuous
prominent
Character 31 Size of the hilar appendix
b
Ramariopsis pulchella
Ramariopsis crocea
Ramariopsis kunzei
Ramariopsis tenuiramosa
Ramariopsis californica
Scytinopogon robustus
Scytinopogon dealbatus
Scytinopogon pallescens
Scytinopogon echinosporus
Clavariadelphus pistilaris
Clavulinopsis laeticolor
Clavulinopsis helvola
Clavulinopsis amoena
Clavulinopsis aurantio-cinnabarina
Clavaria vermicularis
Clavaria sulcata
Clavulinopsis fusiformis
Clavulinopsis corniculata
Clavaria gibbsiae
Clavaria zollingeri
Lactarius indigo
Gomphus floccosus
Gomphus clavatus
equivocal
present
absent
Character 11 Carotenoid pigments

Figure 4. Optimization of two characters used by Petersen (1978a) to define *Ramariopsis*, onto one of the most parsimonious trees. a) Optimization of character 31 showing prominent hilar appendix as a simplesiomorphy; b) optimization of character 11 showing the absence of carotenoid pigments as a symplesiomorphy.

The position of *Clavariadelphus pistillaris* could be an artifact, due to the fact that the present sampling of taxonomic outgroups included distantly related groups –e.g. *Gomphus* in the clade Gomphoide-Phalloide sensu Hibbett & Thorn (2001).

To explore this possibility, a branch and bound search was performed excluding *Clavariadelphus*, and the monophyletic group of *Ramariopsis* sensu Corner+*Scytinopogon* obtained in the main analysis was recovered (results not shown).

It has not been possible to clearly elucidate the phylogenetic affinity of *Clavaria zollingeri*. This taxon was included in the analysis of clavarioid and cantharelloid Homobasidiomycetes by Pine et al. (1999), but their results were inconclusive and this species was located outside of any recognized clade in the strict consensus analysis of combined genes. In spite of this, indicated that the current delimitation of *Clavaria* (Corner 1970) –simple or branched basidiomes, monomitic hyphae in the context, clamp connections absent in the context, and present or absent of clamp connections at the base of the basidia– was not a monophyletic group. The results of our study are congruent with those of Pine et al. (1999).

Clavaria gibbsiae in the present results is located next to *C. zollingeri*, out of any large clade –e.g. *Clavaria* sensu Petersen or *Ramariopsis* sensu Corner. This species is traditionally included in *Clavaria* subgenus *Holocoryne* (Corner 1970, Petersen 1988a). We could expect a relationship between *Clavulinopsis* and representatives of *Clavaria* based on previous results (Pine et al. 1999, Larsson et al. 2004), but these studies also indicate that *Clavaria* is not a monophyletic group (Pine et al. 1999). Our results are congruent with these previous studies and show a core group that includes part of *Clavaria* and all the included representatives of *Clavulinopsis*. These results should be considered as preliminary due to the lack of bootstrap support for this group and the taxonomic sampling of the present study.

One important result from the present study is the proposal of nomenclatural changes in *Ramariopsis*. At the present time, phylogenies seem to be divorced from classifications since few phylogenetic hypotheses are used as the foundation for newer classifications. This may result in the undesirable situation in which robust phylogenetic papers have little impact on the daily practice of taxonomists (for a broader discussion on this issue see Wheeler 2004, Franz 2005). Phylogeneticists are frequently reluctant to introduce changes in the classification due to the nature of the phylogenetic research—e.g. occasionally the relative position of a clade undergoes modifications with the addition of new data. In the present case, we decided to make taxonomical decisions based on our phylogenetic results by introducing changes only when we felt confident to do it.

Our results are robust enough to restrict *Ramariopsis* to species with ornamented spores—which show the characteristic ultrastructural pattern. The only problem arises when we try to identify the species that meet those requirements since in some species spore ornamentation is difficult to see. There are cases in which some taxa originally described with smooth spores are demonstrated to have ornamented spores—one example of this is *Ramariopsis californica* R.H. Petersen (Pegler & Joung 1985). For this reason, and until we have more information—e.g. SEM and TEM studies of the spores—we avoid proposing new combinations for species with smooth spores originally described in *Ramariopsis* (see Appendix 3). We only recommend the use of combinations previously proposed, that are congruent with our results (see Appendix 3). The only exception is *Ramariopsis antillarum* (Pat.) R.H. Petersen. This taxon was originally described as *Clavaria fusiformis* var. *antillarum* Pat.; subsequently, Petersen (1988a) proposed to raise it to species rank based on the differences in the ontogenetic patterns of the basidiomes between yet the species and the variety. We concur with Petersen's proposal, yet we consider that the correct placement for this species is in *Clavulinopsis* based on the presence of simple club basidiomes and globose, smooth spores. Our current knowledge of this species leads us to propose the combination

Clavulinopsis antillarum (Pat.) García-Sandoval & Cifuentes, **comb. nov.**

Basionym: *Clavulinopsis fusiformis* var. *antillarum* Pat., in Duss, Enum. Methodique des champignons recueilles a la Guadeloupe a la Martinique (Lons-le-Saunier): 14 (1903).

We have included a checklist of available species names for *Ramariopsis* and their correct combinations according to the present results (see appendix 3). The list is divided in three parts: a) species confidently placed in *Ramariopsis* sensu stricto; b) taxa once included in *Ramariopsis* that do not belong to *Ramariopsis* according to our results and available information; and c) species originally described in *Ramariopsis* that need further examination before a new combination be proposed. We think that this checklist provides practical applications, avoiding the proposal of unjustifiable new combinations that may result in unstable nomenclatural changes.

Two combinations are excluded from the list:

a) *Ramariopsis bizzozeriana* (Sacc.) Schild. (= *Clavaria bizzozeriana* Sacc.). *C. bizzozeriana* was recognized as a taxonomic synonym of *Ramariopsis pulchella* by Corner (1950); later the combination *Ramariopsis bizzozeriana* was incorrectly preferred over *Ramariopsis pulchella* by Schild (1972). This last combination should not be used because *C. bizzozeriana* is currently considered a taxonomic synonym of *R. pulchella* (for details see Corner 1950, Petersen 1978b).

b) *Ramariopsis lentofragilis* (= *Clavaria lentofragilis* Atk.). Corner (1950 p. 640) considered *Clavaria lentofragilis* Atk. a taxonomic synonym of *Ramariopsis kunzei*, although he kept doubts. In his description of *Ramariopsis lentofragilis* f. *propera* (Bourdot) R.H. Petersen, Petersen (1969 p. 550) used the combination *Ramariopsis lentofragilis* without making any reference to the authority of the combination. In a subsequent article Petersen (1978a p.669) acknowledged Corner as the author of the combination. However, *Ramariopsis lentofragilis* was not considered by Corner (1950, 1970), who only referred to the species as a taxonomic synonym of *R. kunzei*. Whether *C. lentofragilis* is a synonym of *R. kunzei* or not is a matter that needs further investigation. For that reason we prefer to exclude that possible combination from the checklist.

In conclusion, the present study indicates that the delimitation of *Ramariopsis* proposed by Corner (1950, 1970) is robust, given currently available data. Although the topological comparisons did not find significant differences between this hypothesis and that proposed by Petersen (1978a), our analysis indicates that *Ramariopsis* sensu Corner represents a more parsimonious hypothesis (five steps shorter), in accordance with ultrastructural data on spore ornamentation and patterns of cyanophilous reaction in the spores. Additionally, the test of sensitivity of the taxonomic sampling indicated that the results obtained were not an artifact of taxon selection and are stable across various resamplings of the data. Relationships among taxa outside of the clade *Ramariopsis* sensu Corner should be taken as tentative, given that the sampling of the present study was designed for other objectives. Recently Dentinger & McLaughlin (2005) addressed the relationships of *Clavariaceae* and *Pterulaceae*; in their sampling they included representatives of *Ramariopsis* sensu Petersen and *Clavaria* sensu Petersen. Their results agree with our study and show *Ramariopsis* sensu Petersen as a paraphyletic group while also showing support for a clade congruent with *Clavaria* subgenus *Clavulinopsis*. In our results, we also find a clade congruent with the mentioned subgenus of *Clavaria*, but with non-bootstrap support. Future studies addressing *Clavariaceae* question are needed, but current findings (e.g. Dentinger & McLaughlin 2005) provide important insights about this questions.

Acknowledgments

The authors wish to thank Dr. Gregory Mueller of the Field Museum of Chicago and Dra. Laura Guzmán-Dávalos, from Universidad de Guadalajara for peer-review of this manuscript. Also we thank Dr. David S. Hibbett of Clark University in Massachusetts for revision and commentary on a preliminary version of this manuscript; Dr. Ronald H. Petersen of the University of Tennessee for comments and suggestions on a previous version of this work presented at the 2004 Annual Meeting of the Mycological Society of America; Bryn C. Dentinger, of the University of Minnesota, for sharing a copy of their poster presented at the 2005 Annual MSA Meeting; the curators of the herbaria E, ENCB, IBUG, L, MEXU, TENN, TLXM, XAL, especially Dr. Ronald H. Petersen for the facilities and specimens offered, Dr. Roy Watling (E) for the facilities offered for the revision of the collection of Dr. E.J.H. Corner, Dr. Machiel Noordeloos (L) and Evelyn Turnbull (E) for their help during visits to their herbaria. Funds for the present work were provided by CONACYT 34313-V, and DGAPA IN 209605–3. R. G.-S. would also like to thank support from the Programa de Becas Nacionales de Posgrado UNAM. The authors would like to note that conclusions presented in this work are not necessarily those of colleagues that commented earlier versions of this work.

Appendix 1. Characters and character states.

Morphological characters of the basidiome

1.- *Simple clavate basidiome.* This type of basidiome corresponds to what Petersen (1988a) defined as clavarioid –holobasidiomycetes, in simple erect columns– excluding the branched forms which show a distinct ontogenetic pattern as described by Corner (1950). This includes intergradations from simple clavate forms to those with some amount of apical branching (bifurcated towards the apex). Within this general pattern are several ontogenetic variants (Corner 1950, Clémençon et al. 2004) that could be phylogenetically informative, but to date there are too few data to recognize discrete patterns; in the present study only the general pattern was considered. States: 0: present, 1: absent.

2.- *Profusely branched basidiomes.* This character corresponds to what Petersen (1988a) described as clavarioid (see character 1), but is confined to the branched forms, since this corresponds to an ontogenetic pattern distinct from the simple forms (Corner 1950). A basidiome was considered profusely branched when it exhibited three or more levels of branching coming from the middle or below the middle of the basidiome. States: 0: present, 1: absent.

3.- *Basidiome pileate-stipitate.* Corner (1966) defines the pileus as an apical expansion developed from a diageotropic growth that generates fan or umbrella shaped forms. This differs from the cantharelloids in the configuration of the hymenophore and the absence of a thickened hymenium. In the present study any basidiome exhibiting a pileus sensu Corner (1966) and a stipe sensu Kirk et al. (2001) was considered as pileate-stipitate, independent of the conformation of the hymenophore. States: 0: present, 1: absent.

4.- *Form of the hymenophore.* For the present study the hymenophore was defined following Clémençon et al. (2004) as the portion of the context that supports the hymenium –the layer of basidia, basidiospores, and sterile elements– in contrast to the proposal of Kirk et al. (2001), who considered the hymenophore the structure which supports spores –e.g. a basidiome. In the present study the hymenophore was considered to exhibit variation in form independent of that of the basidiome, and as such is an independent character (Mickevich 1982, Mickevich and Limpscomb 1991, Limpscomb 1992, Mabee 1993, O'Keefe & Wagner 2001) –against Clémençon et al. (2004) see character three– for example a smooth hymenophore can be present in a simple, clavate, or corticioid basidiome. The recognized states correspond in the case of gills to the description of Singer (1986), for a hymenophore in folds to Corner (1966) in cantharelloid fungi, for a smooth hymenophore to Clémençon et al. (2004), and for a wrinkled hymenophore to the description of some species of Clavariadelphus by Corner (1950) though with a lesser grade of organization. States 0: gills, 1: folds, 2: wrinkles, 3: smooth.

5.- *Longitudinally sulcate in simple clavate basidiomes.* This character corresponds to the description by Petersen (1988a) and represents those basidiomes that exhibit a furrow or longitudinal fold along the fertile part of the basidiome; it differs from a wrinkled hymenophore in that the furrow or fold is singular. States: 0: present, 1: absent.

6.- *Development of the context at the level of the hymenium.* This character corresponds to the presence or absence of the condition described as fistular or hollow by Kirk et al. (2001), but confined to the context at the level of the hymenium. The portion of the context below the hymenophore and subhymenium can exhibit distinct grades of development with two clearly recognizable states: when it is well developed, the basidiome exhibits a solid aspect in transverse section, but when poorly developed the basidiome appears hollow or fistulate in transverse section. States 0: fistulate, 1: solid.

7.- *Pattern of branching.* Profusely branched basidiomes exhibit different patterns of branching derived from differences in ontogenetic development (Corner 1950). The nomenclature and patterns described by Corner (1950) were followed for the present study. Only two states were observed among the included species in the present analysis. States 0: radial, 1: flattened.

8.- *Mycelial cords.* Aggregates of linear hyphae growing away from the basidiome and visible to the naked eye were considered mycelial cords. Clémençon et al. (2004) and Boddy (1999) distinguished between mycelial cords and rhizomorphs based on the level of organization and the type of growth of the structure. In the present study no distinctive apical growth was identified, and the observed structures were thus only characterized as mycelial cords in the general sense of Cairney et al. (1991). States: 0: present, 1: absent.

9.- *Mycelium at the base of the stipe.* Basidiomes growing from a patch or pillow of mycelium were considered as exhibiting mycelia at the base of the stipe. The mycelial growth was always conspicuous and found above the substrate; this mycelium covers the base of the stipe and exhibits different types of generative hyphae. Petersen (1988a) described subiculate as a patch of mycelia in the substrate from where the basidiome grows, but Clémençon et al. (2004) restricted the term subiculate to the thick layer of mycelia from which the corticioid basidiomes develop. We treat the character as equivalent to what Petersen (1988a) denominates subiculate, but since Clémençon et al. (2004) employed the term in a different manner, the descriptor subiculate is not used in the present study. States: 0: present, 1: absent.

10.- *Reaction of hymenium to iron salts.* The reaction to iron salts is a widely used character in the systematics of clavarioid fungi (Corner 1950; Donk 1964; Petersen 1978a, 1988a). The reagent contains ferric chloride in a 10% aqueous solution (Petersen 1988a), and is applied directly to the hymenium. A positive reaction is recognized by a color change to olive-green or gray-green. This reaction is considered indicative of the presence of the compound pistillarine (Steglich et al. 1984). A positive reaction to this reagent can exhibit other color changes due to the presence of distinct compounds (Gill & Steglich 1987, Singer 1986). In the present study only positive reactions that engendered olive-green or gray-green color changes were considered. Observations were made in dryed exemplars. In our experience species with positive reaction in fresh material also react when dry. States 0: positive, 1: negative.

11.- *Carotenoid pigments in the basidiome.* Along with sesquiterpinoids, carotenoids are the only pigments present in the macromycetes derived from the mevalonate pathway (Gill & Steglich 1987, Gill 2003). This character was coded as a nominal variable –sensu Hawkins (2000)– based on the aviable information for the species considered since other types of coding would require additional data about the specific metabolic pathways generating the compound (e.g. Barkman 2001). States 0: present, 1: absent.

Micromorphological characters distinct from the hymenium and the spores

12.- *Lacticiferous hyphae.* This structure corresponds to what Singer (1986) described as lacticiferous in the strict sense –hyphae that produce latex. These hyphae can exhibit nuclei and septa and thus correspond to a specialized type of heteroplera sensu Clémençon et al. (2004). States 0: present, 1: absent.

13.- I*nflated hyphae.* Inflated hyphae are those generative hyphae that exhibit increased growth behind the point of lateral growth, widening and elongating significantly (Corner 1950, Kirk et al. 2001). They are recognizable by having a considerably greater diameter than the rest of the hyphae, and by having constrictions in the zone of the septa; they may or may not have clamp connections. Corner (1950) distinguished two types of monomitic contexts that present inflated hyphae based on the presence of secondary septa and clamps. In the present study this classification was not used, since it mixes two independently varying characters. States: 0: present, 1: absent.

14.- *Crystals in the hyphae of the basal mycelia.* The hyphae of the mycelia at the base of the stipe sometimes present amorphous crystals, similar to those reported in the context of the base of the stipe for *Ramariopsis pulchella* (Petersen 1988a) –5-20μm, hyaline or yellowish, and do not dissolve in 5% KOH. The crystals are found covering the exterior surface of the hyphae and are not easily removed. States: 0: present, 1: absent.

15.- *Degree of thickening in the hyphal wall.* Thickening of the hyphal wall was considered only in the generative hyphae. The presence of generative hyphae with thickened walls has been a relevant systematic character in several genera (Corner 1966, Pegler 1996). In the present study three qualitative degrees of thickening were recognized due to the difficulty in making precise quantitative measurements. States: 0: none, 1: scarce, 2: conspicuous.

16.- *Simple fibulae.* Simple fibulae –clamp connections, clamp cells– are frequently observed in Basidiomycetes. Clémençon et al. (2004) recognized three types of simple fibulae, of which two were observed in the present study: closed and ring or medallion fibulae. Both types were considered equivalent since there were insufficient elements to determine discrete states, due the continuous variation observed. States: 0: present, 1: absent.

17.- *Geniculate fibulae.* Geniculate fibulae are those that exhibit a marked bend at the point of inflection, giving the appearance of a bent knee. The bend partially deforms the profile of the fibulae, allowing them to be easily differentiated from simple fibulae. These structures correspond to those described in various species of *Clavulinopsis* by Petersen (1968). Geniculate fibulae are not homologous to simple fibulae since both structures are found simultaneously in the same basidiome and are thus independent characters according to the conjunction test (Patterson 1988, Rieppel 1988, De Luna & Mishler 1996, Rieppel & Kearney 2002, Grant & Kluge 2004). States: 0: present, 1: absent.

18.- *H connections.* These connections are structures formed by the union of two parallel hyphae through a third, transverse, hypha. They can be considered functional homologous –biologically homologous following Roth (1988)– to fibulae, but are not phylogenetically homologous sensu de Pinna (1991), since they are both present simultaneously with distinct types of fibulae. They should thus be considered independent characters following the conjunction test (Patterson 1988, Rieppel 1988, De Luna & Mishler 1996, Rieppel & Kearney 2002, Grant & Kluge 2004). States: 0: present, 1: absent.

19.- *Ampulliform fibulae.* This type of fibula is characterized by the presence of a marked widening, giving the appearance of an inflated fibula similar to the inflated hyphae. This corresponds to the description by Petersen (1988a) as a characteristic of *Ramaria* subg. *Lentoramaria* Corner. This type of fibula is not phylogenetically homologous to the other types of fibulae described since it can be found present simultaneously with those other structures and should therefore be considered as an independent character by the conjunction test (Patterson 1988, Rieppel 1988, De Luna & Mishler 1996, Rieppel & Kearney 2002, Grant & Kluge 2004). States: 0: present, 1: absent.

Hymenial characters

20.- *Subhymenium clearly differentiated.* The subhymenium was considered to be the layer of generative hyphae growing below the hymenium (Kirk et al. 2001) and from which the hymenium forms (Petersen 1988a). Several distinct anatomical patterns of the subhymenium have been described (Clémençon et al. 2004), and in the case of the clavarioid fungi, Petersen (1988a) considered three types. The variation observed in the present study; did not permit differentiation of distinct types of subhymenium; only the conspicuous presence or absence of a subhymenium was considered. States: 0: present, 1: absent.

21.- *Thickening of the hymenium.* Corner (1950) described the thickening of the hymenium as a pattern resulting from the sympodial growth of the hyphae of the subhymenium, generating successive superimposed layers of hymenium, collapsing the preceding basidia. No additional patterns of variation of this type have been described to date, and in the present study no further variation was observed. States: 0: present, 1: absent.

22.- *Cystidia of the hymenium.* Cystidia are sterile hyphal apices, generally with a distinctive form and found in variable locations in the basidiome (Kirk et al. 2001, Clémençon et al. 2004). The form and anatomic disposition of the cystidia have been used as taxonomic characters, but only the cystidia in the hymenium were considered for the present study, as no other cystidia were observed in the species studied. States: 0: present, 1: absent.

23.- *Fibulate basidia.* Clamp connections on basidia are restricted to the base of the basidia. The presence on the basidia is independent of the presence of fibulae in the rest of the hyphae of the basidiome. As such, it was considered as an independent character. In the case of *Clavaria* subgenus *Holocoryne* the basidia exhibit a fibula described as broadly free (Corner 1950) or bifurcated (Petersen 1988a). States: 0: present, 1: absent.

24.- *Basidia with refringent contents.* This character refers to basidia with an oily, yellowish, appearance of its content, which is homogeneous and refringent in 10% KOH. It corresponds partially to what Petersen (1988a) described as gloeoplerotic, excluding the foamy appearance. Similarly, it corresponds partially to what Clémençon et al. (2004) described as oil-producing –resinous content– and to what Singer (1986) describes as oil-producing sensu Fayoid, but without the positive sulfovainillin reaction. States: 0: present, 1: absent.

25.- *Shape of the basidia.* The variation observed during the present study permitted recognition of two forms or general profiles of basidia: cylindrical and clavate. These terms correspond to the definitions of Kirk et al. (2001). States 0: clavate, 1: cylindrical.

26.- *Base of clavate basidia.* Clavate basidia exhibit variation in the size of the base. Variation of the size was coded as an independent character because it refers to a property or feature of an anatomical region particular to clavate basidia and is not homologous with terete basidia (de Pinna 1991, De Luna & Mishler 1996, Rieppel & Kearney 2002, Grant & Kluge 2004). Additionally, this coding reflects the variation observed as it describes properties with independent variation. The size of the basidia exhibits variation logically independent of the form and thus can be coded as an independent character (Hawkins et al. 1997, Hawkins 2000, O'Keefe & Wagner 2001, Rieppel & Kearney 2002). States 0: short, 1: long.

27.- *Geniculate basidia.* These basidia exhibit a point of inflection in the middle part, and thus a marked bend giving the appearance of a flexed knee. This bend conspicuously deforms the profile of the basidia allowing them to be clearly differentiated. This pattern corresponds to that described in various species of *Clavulinopsis* by Petersen (1968). Geniculate basidia do not constitute a pattern or form homologous in the phylogenetic sense (de Pinna, 1991), since both structures are simultaneously present in the same basidiome and thus constitute independent characters by the conjunction test (Patterson 1988, Rieppel 1988, De Luna & Mishler 1996, Rieppel & Kearney 2002, Grant & Kluge 2004). States: 0: present 1: absent.

28.- *Orientation of the achromatic spindle.* Juel (1898) described two basic patterns of orientation of the meiotic spindle of basidia during meiosis: chiastic –transversal to the principal axis and situated in the apex– and stictic –parallel to the principal axis and situated in the middle. Boidin (1958) recognized an intermediate pattern that he called hemichiastic; Donk (1964) later qualified this as a homologous variant of the chiastic form. In the present study only the chiastic and stictic patterns were considered, given that those are the only patterns reported for the species studied. States: 0: chiastic, 1: stictic.

29.- *Four remaining nuclei.* After meiosis a third nuclear division sometimes occurs, producing a total of eight nuclei. In some species four of these nuclei disintegrate and are termed remaining nuclei (Penancier 1961). This pattern has been reported for several of the species considered in the present study and corresponds to that described as post-meiotic pattern "A" by Duncan & Galbraith (1972) and to that described by Kühner (1977). The data available for the species considered in the present study only allowed recognition of the presence of a third division –exhibiting pattern "A"– and the absence of this division (only four nuclei form); this character was thus coded as a nominal variably (Hawkins 2000). States: 0: present, 1: absent.

Characters of the basidiospores

30.- *Spore form.* The variation observed during the present study allowed the recognition of three spore forms: globose, subglobose, and elongate. States were assigned qualitatively, excluding the deformations produced by ornamentation when present, and correspond to the forms described by Kirk et al. (2001), except for the fusiform and ellipsoid forms, which are considered as elongated spores. States: 0: subglobose, 1: globose, 2: elongate.

31.- *Size of the hilar appendix.* The hilar appendix –also called the apicule, sterigmal appendix or apophysis (Kirk et al. 2001, Clémençon et al. 2004)– is the small conical or papilla-shaped projection, which is the point of connection between the spore and the sterigma. This structure is involved in the active liberation of the spores (Clémençon et al. 2004). Based on the observed variation in the species studied, two qualitative states were recognized to describe the size of the appendix. States: 0: prominent, 1: inconspicuous.

32.- *Thickness of the spore wall.* The species considered in the present study do not exhibit significantly thickened spore walls, but some species show a slight thickening. Based on the observed variation two qualitative states were recognized. States: 0: slightly thickened, 1: thin.

33.- *Smooth spores.* Spore ornamentation has been a relevant taxonomic character for *Ramariopsis* (Corner 1950), but several studies have shown that ornamentation can be derived from different layers of the spore wall. Treating the presence of ornamentation as homologous in different taxa could thus fail the test of similarity (Rieppel 1988, Nelson 1994, Rieppel & Kearny 2002). The presence of smooth spores –spores without modifications or deformations in the wall– was observed in preparations mounted in 5% KOH using a bright field light microscope at 1000x magnification. States: 0: present, 1: absent.

34.- *Ultrastructure of spore ornamentation.* The spore wall has been characterized in different studies using different sources of information –light and electron microscopy– that generated different terms to denominate the observed ultrastructural patterns (Clémençon et al. 2004). It is known that seemingly similar forms can exhibit different ultrastructural patterns (Clémençon et al., 2004), and as such regarding these forms as homologous sensu de Pinna (1991) would be incorrect by the test of similarity (Rieppel & Kearney 2002, Grant & Kluge 2004). Based on this knowledge, the ultrastructure of the ornamentation was coded instead by the morphological patterns observed. In the present study the nomenclature of Pegler & Young (1985) was used, which also corresponds to the descriptions of Hawksworth et al. (1995). States: 0: tunica, 1: corium.

35.- *Cyanophilous reaction of the spores.* For the present study a cyanophilous reaction was considered positive when the wall of the spore stains with cotton blue, following the nomenclature proposed by Kotlaba & Pouzar (in Donk 1964). During the present study the reagent was prepared dissolving 1.6 g of cotton blue in 10 ml of lactic acid. After adding the reagent, the preparation was heated until boiling and then left to cool for 10 minutes before observations were made. States: 0: positive, 1: negative.

36.- *Pattern of cyanophylly in the spores.* The positive reaction to cotton blue (cyanophylly) exhibits two patterns of coloring: homogeneous or more intense in the ornamentation. States: 0: homogeneous coloring, 1: ornamentation more cyanophilous.

Appendix 2. Data matrix

Table 2. Data matrix for the 23 species and 36 characters.

? = missing data, – = inapplicable data. For description of the characters and character states see Appendix 1.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Clavaria amoena	0	1	1	3	0	1	–	1	0	0	0	1	0	1	1	0	0	0
Clavaria aurantiocinnabarina	0	1	1	3	0	1	–	1	0	1	0	1	0	0	1	0	0	0
Clavaria gibbsiae	0	1	1	3	0	0	–	1	0	1	?	1	0	?	1	1	–	0
Clavaria sulcata	0	1	1	3	0	1	–	1	1	1	?	1	0	–	1	0	0	0
Clavaria vermicularis	0	1	1	3	1	1	–	1	1	1	?	1	0	–	1	1	–	0
Clavaria zollingeri	1	0	1	3	–	0	0	1	0	1	?	1	1	0	1	1	–	0
Clavariadelphus pistillaris	0	1	1	2	1	0	–	1	0	0	1	1	0	0	1	0	1	0
Clavulinopsis corniculata	1	0	1	3	–	0	0	1	0	0	1	1	0	0	1	0	0	0
Clavulinopsis fusiformis	0	1	1	3	0	1	–	1	0	0	1	1	0	1	1	0	1	0
Clavulinopsis helvola	0	1	1	3	0	1	–	1	0	1	?	1	0	1	1	0	0	?
Clavulinopsis laeticolor	0	1	1	3	0	1	–	1	0	0	1	1	0	?	1	0	0	0
Gomphus clavatus	1	1	0	1	–	0	–	0	1	1	1	1	1	–	0	0	1	?
Gomphus floccosus	1	1	0	1	–	0	–	0	0	1	?	1	1	0	0	1	–	?
Lactarius indigo	1	1	0	0	–	0	–	1	1	1	?	0	1	–	1	1	–	1
Ramariopsis californica	1	0	1	3	–	1	0	1	0	1	?	1	0	1	1	0	1	0
Ramariopsis crocea	1	0	1	3	–	0	0	1	0	1	1	1	0	1	1	0	1	0
Ramariopsis kunzei	1	0	1	3	–	0	0	1	1	1	?	1	0	–	1	0	1	0
Ramariopsis pulchella	1	0	1	3	–	0	0	1	0	1	?	1	0	1	1	0	1	0
Ramariopsis tenuiramosa	1	0	1	3	–	0	0	1	1	1	?	1	0	–	1	0	1	0
Scytinopogon dealbatus	1	0	1	3	–	0	1	1	1	1	?	1	1	–	2	0	1	0
Scytinopogon echinosporus	1	0	1	3	–	0	1	0	1	1	?	1	1	–	2	0	1	0
Scytinopogon robustus	1	0	1	3	–	0	1	0	0	1	?	1	1	0	1	0	1	0
Scytinopogon pallescens	1	0	1	3	–	0	1	1	1	1	?	1	1	–	2	0	1	0

Table 2, continued

	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36
Clavaria amoena	1	0	0	0	0	0	0	1	0	0	1	2	1	1	0	?	1	–
Clavaria aurantiocinnabarina	1	1	0	1	0	0	0	1	1	0	1	0	1	1	0	?	1	–
Clavaria gibbsiae	–	0	1	1	0	0	0	1	1	?	?	2	0	1	1	?	1	–
Clavaria sulcata	1	0	0	1	0	1	0	1	1	?	?	2	1	1	0	?	1	–
Clavaria vermicularis	–	0	0	1	1	1	0	1	1	?	?	2	1	1	0	?	1	–
Clavaria zollingeri	–	0	0	1	1	1	0	1	1	?	?	2	1	1	0	?	1	–
Clavariadelphus pistillaris	0	0	0	1	0	1	0	1	1	0	?	2	1	0	0	?	1	–
Clavulinopsis corniculata	1	0	0	1	0	1	0	1	1	0	0	1	0	0	0	?	1	–
Clavulinopsis fusiformis	1	0	0	0	0	0	0	1	0	0	0	1	0	0	0	?	1	–
Clavulinopsis helvola	1	0	0	1	0	0	0	1	0	?	?	0	0	0	1	1	0	0
Clavulinopsis laeticolor	1	0	0	1	0	0	0	1	0	0	0	2	0	0	0	?	1	–
Gomphus clavatus	1	0	0	1	0	1	0	1	1	0	?	2	0	1	1	?	0	1
Gomphus floccosus	–	0	0	1	1	1	0	1	1	?	?	2	0	1	1	?	0	1
Lactarius indigo	–	0	1	0	1	1	0	1	1	?	?	2	0	1	1	?	0	0
Ramariopsis californica	1	0	0	1	0	1	0	0	1	?	?	0	0	1	1	0	0	1
Ramariopsis crocea	1	0	0	1	0	1	0	0	1	0	0	0	0	0	1	0	0	1
Ramariopsis kunzei	1	0	0	1	0	1	0	0	1	?	?	0	0	0	1	0	0	1
Ramariopsis pulchella	1	0	0	1	0	1	0	0	1	?	?	0	0	0	1	0	0	1
Ramariopsis tenuiramosa	1	0	0	1	0	1	0	0	1	?	?	0	0	0	1	0	0	1
Scytinopogon dealbatus	1	0	0	1	0	1	1	–	1	?	?	0	1	0	1	?	0	0
Scytinopogon echinosporus	1	0	0	1	0	1	1	–	1	?	?	2	1	0	1	?	0	0
Scytinopogon robustus	1	0	0	1	0	1	1	–	1	?	?	0	1	0	1	?	0	0
Scytinopogon pallescens	1	0	0	1	0	1	1	–	1	?	?	0	1	0	1	?	0	0

Appendix 3. Checklist of species names.

Part 1. Species of *Ramariopsis* sensu stricto

Ramariopsis asterella (G.F. Atk) Corner
  ≡ *Clavaria asterella* G.F. Atk.
Ramariopsis avellanea R.H. Petersen
Ramariopsis avellaneainversa R.H. Petersen
Ramariopsis biformis (G.F. Atk.) R.H. Petersen
  ≡ *Clavaria biformis* G.F. Atk.
Ramariopsis californica R.H. Petersen
Ramariopsis cinnamomea R.H. Petersen
Ramariopsis cinnamomipes R.H. Petersen
Ramariopsis citrina Schild
Ramariopsis clavuligera (R. Heim) Corner
  ≡ *Clavaria clavuligera* R. Heim
Ramariopsis costaricensis L.D. Gómez
Ramariopsis crocea (Pers.) Corner
  ≡ *Clavaria crocea* Pers.
Ramariopsis curta (Fr.) Corner
  ≡ *Clavaria curta* Fr.
Ramariopsis flavescens R.H. Petersen
Ramariopsis hibernica Corner
Ramariopsis kunzei (Fr.) Corner
  ≡ *Clavaria kunzei* Fr.
Ramariopsis longipes R.H. Petersen
Ramariopsis novahibernica Corner
Ramariopsis pulchella (Boud.) Corner
  ≡ *Clavaria pulchella* Boud.
Ramariopsis ramarioides R.H. Petersen
Ramariopsis rufipes (G.F. Atk.) R.H. Petersen
  ≡ *Clavaria rufipes* G.F. Atk.
Ramariopsis subarctica Pilát
Ramariopsis tenuicula (Bourdot & Galzin) R.H. Petersen
  ≡ *Clavaria tenuicula* Bourdot & Galzin
Ramariopsis tenuiramosa Corner
Ramariopsis tortuosa R.H. Petersen
Ramariopsis vestitipes (Peck) Corner
  ≡ *Clavaria vestitipes* Peck

Part 2. Species sometimes placed in *Ramariopsis* that belong in other genera

Clavaria L.

Clavaria asperulospora G.F. Atk.
  ≡ *Ramariopsis asperulospora* (G.F. Atk.) Corner

Clavulinopsis Overeem

Clavulinopsis antillarum (Pat.) García-Sandoval & Cifuentes, comb. nov.
  ≡ *Clavaria fusiformis* var. *antillarum* Pat.
  ≡ *Ramariopsis antillarum* (Pat.) R.H. Petersen
Clavulinopsis corniculata (Schaeff.) Corner
  ≡ *Clavaria corniculata* Schaeff.

≡ *Ramariopsis corniculata* (Scaeff.) R.H. Petersen
Clavulinopsis depokensis (Overeem) Corner
≡ *Clavaria depokensis* Overeem
≡ *Ramariopsis depokensis* (Overeem) R.H. Petersen
Clavulinopsis dichotoma (Godey) Corner
≡ *Clavaria dichotoma* Godey
≡ *Ramariopsis dichotoma* (Godey) R.H. Petersen
Clavulinopsis fusiformis (Sowerby) Corner
≡ *Clavaria fusiformis* Sowerby
≡ *Ramariopsis fusiformis* (Sowerby) R.H. Petersen
Clavulinopsis helvola (Pers.) Corner
≡ *Clavaria helvola* Pers.
≡ *Ramariopsis helvola* (Pers.) R.H. Petersen
Clavulinopsis holmskiodii (Oudem.) Corner
≡ *Clavaria holmskiodii* Oudem.
≡ *Ramariopsis holmskiodii* (Oudem.) R.H. Petersen
Clavulinopsis laeticolor (Berk. & M.A. Curtis) R.H. Petersen
≡ *Clavaria laeticolor* Berk. & M.A. Curtis
≡ *Ramariopsis laeticolor* (Berk. & M.A. Curtis) R.H. Petersen
Clavulinopsis luteo-ochracea (Cavara) Corner
≡ *Clavaria luteo-ochracea* Cavara
≡ *Ramariopsis luteo-ochracea* (Cavara) R.H. Petersen
Clavulinopsis luteotenerrima (Overeem) Corner
≡ *Clavaria luteotenerrima* Overeem
≡ *Ramariopsis luteotenerrima* (Overeem) R.H. Petersen
Clavulinopsis minutula (Bourdot & Galzin) Corner
≡ *Clavaria minutula* Bourdot & Galzin
≡ *Ramariopsis minutula* (Bourdot & Galzin) R.H. Petersen
Clavulinopsis subtilis (Pers.) Corner
≡ *Clavaria subtilis* Pers.
≡ *Ramariopsis subtilis* (Pers.) R.H. Petersen
Clavulinopsis umbrinella (Sacc.) Corner
≡ *Clavaria umbrinella* Sacc.
≡ *Ramariopsis umbrinella* (Sacc.) R.H. Petersen

Ramaria Fr.

Ramaria lorithamnus (Berk.) R.H. Petersen
≡ *Clavaria lorithamnus* Berk.
≡ *Ramariopsis lorithamnus* (Berk.) Corner

Scytinopogon Singer

Scytinopogon dealbatus (Berk.) Corner
≡ *Clavaria dealbata* Berk.
≡ *Ramariopsis dealbata* (Berk.) R.H. Petersen

Part 3. Species initially described in *Ramariopsis* that require further examination

Ramariopsis agglutinata R.H. Petersen
Ramariopsis alutacea R.H. Petersen
Ramariopsis aurantio-olivacea R.H. Petersen
Ramariopsis bicolor R.H. Petersen
Ramariopsis cremicolor R.H. Petersen

Ramariopsis junquillea R.H. Petersen
Ramariopsis lignicola R.H. Petersen
Ramariopsis ovispora R.H. Petersen
Ramariopsis pseudosubtilis R.H. Petersen
Ramariopsis simplex R.H. Petersen

References

Arpin N, Fiasson J-L. 1971. The pigments of basidiomycetes: their chemiotaxonomic interest. In: Petersen RH (ed.). Evolution in the higher Basidiomycetes: An international symposium. The University of Tennessee Press, Knoxville.

Barkman TJ. 2001. Character coding of secondary chemical variation for use in phylogenetic analyses. Biochem. Syst. Ecol. 29: 1-20.

Bataile F. 1969. Les reactions macrochimiques chez les champignons. J. Cramer, Vaduz.

Bertagnolli R, Novello LA. 2004. Su alcune *Clavariaceae* interessanti. Rev. Mycol. 48: 3-24.

Binder M, Hibbett DS. 2002. Higer-level phylogenetic relationships of Homobasidiomycetes (mushroom forming fungi) inferred from four rDNA regions. Mol. Phyl. Evol. 22: 76-90.

Boidin J. 1958. Essai biotaxonomique sur les hydnés résupinés et les corticiés: etude spéciale du comportement nucléaire et des mycéliums. Rev. Mycol. Mém. 6: 1-387.

Boddy L. 1999. Saprophytic cord-forming fungi: meeting the challenge of heterogeneous environments. Mycologia 91: 13-32.

Bremer K. 1994. Branch support and tree stability. Cladistics 10: 295-304.

Brower AVZ, Schawaroch V. 1996. Three steps in homology assessment. Cladistics 12: 265-272.

Cairney JWG, Jennings DH, Agerer R. 1991. The nomenclature of fungal multi-hyphal linear aggregates. Crypt. Bot. 2: 246-251.

Carpenter JM. 1988. Choosing among equally parsimonious cladograms. Cladistics 4: 291-296.

Carpenter JM. 1994. Successive weighting, reliability and evidence. Cladistics 10: 215-220.

Claus G. 1983. L' ancient genere *Clavaria* Fr. (ss. lato B.-C.). Docs. Mycol. 13: 17-43.

Clémençon H, Emmett V, Emmett EE. 2004. Cytology and plectology of the Hymenomycetes. J. Cramer, Berlin.

Coker WC. 1923. The clavarias of the United States and Canada. The University of North Carolina Press, North Carolina.

Corner EJH. 1950. A monograph of *Clavaria* and allied genera. Ann. Bot. Memm. 1: 1-740.

Corner EJH. 1957. Some clavarias from Argentina. Darwiniana 11: 193-206.

Corner EJH. 1966. A monograph of cantharelloid fungi. Oxford University Press, Oxford.

Corner EJH. 1967a. Clavarioid fungi of Solomon Islands. Proc. Linnean Soc. Lond. 178: 91-106.

Corner EJH. 1967b. Notes on *Clavaria*. Trans. Brit. Mycol. Soc. 50: 33-44.

Corner EJH. 1970. Supplement to "A monograph of *Clavaria* and allied genera". J. Cramer, Stuttgart.

De Luna E, Mishler B. 1996. El concepto de homología filogenética y la selección de caracteres taxonómicos. Bol. Soc. Bot. Mex. 59: 131-146.

De Pinna MCC. 1991. Concepts and test of homology in the cladistic paradigm. Cladistics 7: 367-394.

DeBry RW, Olmstead RG. 2000. A simulation study of reduced tree-search effort in bootstrap resampling analysis. Syst. Biol. 49: 171-179.

Dentinger BC, McLaughlin DJ. 2005. Homoplastic surgery: reconstructing the classification of two coral mushroom families (*Clavariaceae* and *Pterulaceae*) Inoculum 56: 16.

Donk MA. 1933. Revision der Niederländischen Homobasidiomycetae–*Aphyllophoraceae* II. Mededeel. Bot. Mus. Herb. Rijksuniv. Utrecht 9: 1-278.

Donk MA. 1954. The generic names proposed for Hymenomycetes III. *Clavariaceae*. Reinwardtia 2: 441-493.

Donk MA. 1964. A conspectus of the families of *Aphyllophorales*. Persoonia 3: 199-324.

Duncan EG, Galbraith MH. 1972. Post-meiotic events in the Holobasidiomycetidae. Trans. Brit. Mycol. Soc. 60: 463-470.

Eriksson T. 1999. AutoDecay, version 4.0. Bergius Foundation, Royal Academy of Sciences.

Fiasson J-L, Petersen RH, Bouchez M-P, Arpin N. 1970. Contribution biochimique à la connaissance taxonomique des certains champignons cantareloïdes et clavarïoides. Rev. Mycol. 34: 357-364.

Farris JS. 1969. A successive approximation approach to character weighting. Syst. Zool. 18: 374-385.

Farris JS. 1983. The logical basis of phylogenetic analysis. In Platnic NI, Funk VA (eds.). Advances in cladistics, Volume 2. Columbia University Press, New York.

Farris JS. 1991. Hennig defined paraphyly. Cladistics 7: 297-304.

Feibelman TP, Doudrick RL, Cibula WG, Bennett JW. 1997. Phylogenetic relationships within the *Cantharellaceae* inferred from sequence analysis of the nuclear large subunit rDNA. Mycol. Res. 101: 1423-1430.

Felsenstein J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39: 738-791.

Franz NM. 2005. On the lack of good scientific reasons for the growing phylogeny/ classification gap. Cladistics 21: 495-500.

Frisvad JC, Bridge PD, Arora DK. 1998. Chemical fungal taxonomy. Marcel Dekker, New York.

Gill M. 2003. Pigments of fungi (Macromycetes). Nat. Prod. 20: 615-639.

Gill M, Steglich W. 1987. Pigments of fungi (Macromycetes). Prog. Chem. Org. Nat. Prod. 51: 1-317.

García-Sandoval R, Villegas M, Cifuentes J. 2002. New records of *Ramariopsis* from Mexico. Mycotaxon 82: 323-333.

Gómez LD. 1972. Fungi costarricense nonnulli, I.: "*Clavariaceae*" novae. Darwiniana 17: 393-396.

Grant T, Kluge AG. 2003. Data exploration in phylogenetic inference: scientific, heuristic, or neither. Cladistics 19: 379-418.

Grant T, Kluge AG. 2004. Transformation series as an ideographic character concept. Cladistics 20: 23-31.

Hansen L, Knudsen H. 1997. Nordic macromycetes. Vol. 3. Heterobasidioid, Aphyllophoroid and Gasteromycetoid Basidiomycetes. Nordsvamp, Copenhagen.

Hauser DL, Presch W. 1991. The effect of ordered characters on phylogenetic reconstruction. Cladistics 7: 243-265.

Hawkins JA. 2000. A survey of primary homology assessment: different botanist perceive and define characters in different ways. In Scotland R, Pennington RT (eds.). Homology and Systematics. Taylor & Francis, London.

Hawkins JA, Hughes CE, Scotland RW. 1997. Primary homology assessment, characters and character states. Cladistics 13: 275-283.

Hawksworth DL, Kirk PM, Sutton BC, Pegler DN. 1995. Ainsworth & Bisby's Dictionary of the Fungi. 8th ed. International Mycological Intitute, CAB International, Wallingford.

Hennig W. 1966. Phylogenetic systematics. University of Illinois Press, Urbana.

Hibbett DS, Pine E, Langer E, Langer G, Donoghue MJ. 1997. Evolution of gilled mushrooms and puffballs inferred from ribosomal DNA sequences. Proc. Nati. Acad. Sci. USA 94: 12002-12006.

Hibbett DS, Thorn RG. 2001. Basidiomycota: Homobasidiomycetes. In McLaughlin DJ, McLaughlin EG, Lemke PA (eds.). The Mycota VII. Systematics and Evolution. Part B. Springer, Berlin.

Hopple JSJ, Vilgalys R. 1999. Phylogenetic relationships in the mushroom genus *Coprinus* and dark-spored based on sequence data from the nuclear gene coding for the large ribosomal subunit RNA: divergent domains, outgroups, and monophyly. Mol. Phylogen. Evol. 13: 1-19.

Hubbard M, Petersen RH. 1979. Studies in basidial nuclear behavior of selected species of clavarioid and cantharelloid fungi. Sydowia 8:209-223.

Humpert AJ, Muench EL, Giachini AJ, Castellano MA, Spatafora JW. 2001. Molecular phylogenetics of *Ramaria* and related genera: evidence from nuclear large subunit and mitochondrial small subunit rDNA sequences. Mycologia 93: 465-477.

Jenner RA. 2004. Accepting partnership by submission? Morphological phylogenetics in a molecular millennium. Syst. Biol. 53: 333-342.

Juel HO. 1898. Die kernteilungen in den basidien un die phylogenie der Basidiomyceten. Jahrb. Wiss. Bot. 32: 361-388.

Jülich W. 1981. Higher taxa of the Basidiomycetes. J. Cramer, Leiden.

Jülich W. 1985. Bemerkungen zu *Clavulinopsis* und *Ramariopsis*. Int. J. Mycol. Lichenol. 2: 119-122.

Kirk PM, Cannon PF, David JD, Stalpers JA. 2001. Ainsworth & Bisby´s Dictionary of the Fungi. 9th ed. International Mycological Institute, CAB International, Wallingford.

Kluge AG. 2003. The repugnant and the mature in phylogenetic inference: atemporal similarity and historical identity. Cladistics 19: 356-368.

Kühner R. 1977. Variation of nuclear behaviour in the Homobasidiomycetes. Trans. Brit. Mycol. Soc. 68: 1-16.

Larsson KH, Larsson E, Koljalg U. 2004. High phylogenetic diversity among corticioid homobasidiomycetes. Mycol. Res. 108: 983-1002.

Limpscomb DL. 1992. Parsimony, homology and the analysis of multistate characters. Cladistics 8:45-65.

Mabee PM. 1993. Phylogenetic interpretation of ontogenetic change: sorting out the actual and artefactual in an empirical case study of centrarchid fishes. Zool. J. Linnean Soc. 107: 175-291.

Mickevich MF. 1982. Transformation series analysis. Syst. Zool. 31: 461-478.

Mickevich MF, Limpscomb D. 1991. Parsimony and the choice between different transformations for the same character set. Cladistics 7: 111-139.

Mort ME, Soltis PS, Soltis DE, Mabry ML. 2000. Comparison of three methods for estimating internal support on phylogenetic trees. Syst. Biol. 49: 160-171.

Mueller GM, Ammirati JF. 1993. Cytological studies in *Laccaria* (Agaricales). II. Assesing phylogenetic relationships among *Laccaria*, *Hydnangium*, and other Agaricales. Am. J. Bot. 80: 322-329.

Nelson G. 1994. Homology and systematics: the hierarchical basis of comparative biology. In Hall BK (ed.). Homology. Academic Press, San Diego.

Nixon KC. 2002. WinClada ver 1.00.08 Published by the author, Ithaca.

Nixon KC, Carpenter JM. 1993. On outgroups. Cladistics 9: 413-426.

O'Keefe FR, Wagner PJ. 2001. Inferring and testing hypotheses of cladistic character dependence by using character compatibility. Syst. Biol. 50: 657-675.

Patterson C. 1988. Homology in classical and molecular biology. Mol. Biol. and Evol. 5: 603-625.

Pegler DN, Young WK. 1985. Basidiospore structure in *Ramariopsis* (*Clavariaceae*). Trans. Brit. Mycol. Soc. 84: 207-214.

Pegler DN. 1996. Hyphal analysis of basidiomata. Mycol. Res. 100: 129-142.

Penancier N. 1961. Recherches sur l'orientation des fuseaux mitotiques dans la baside des *Aphyllophorales*. Trav. Lab. La Jaysinia 2: 57-71.

Petersen RH. 1964. Notes on clavarioid fungi. II. Corrections in the genera *Ramariopsis* and *Clavaria*. Bull. Torr. Bot. Club 91: 274-280.

Petersen RH. 1965. Notes on clavarioid fungi. IV. Nomenclature and synonymy of *Clavulinopsis pulchra* and *Clavaria laeticolor*. Mycologia 58: 201-207.

Petersen RH. 1966. Notes on clavarioid fungi. V. Emendation and additions to *Ramariopsis*. Mycologia 58: 201-207.

Petersen RH. 1967. Type studies in *Clavariaceae*. Sydowia 21: 105-122.

Petersen RH. 1968. The genus *Clavulinopsis* in North America. Mycologia Memm. 2: 1-40.

Petersen RH. 1969. Notes on clavarioid fungi. X. New species and type studies in *Ramariopsis*, with a key to species in North America. Mycologia 61: 549-559.

Petersen RH. 1971a. Notes on clavarioid fungi. IX. Addendum to *Clavulinopsis* in North America. Persoonia 6: 219-229.

Petersen RH. 1971b. Type studies on clavarioid fungi IV. Specimens from Herbarium Fries at Uppsala. Friesia 9: 369-388.

Petersen RH. 1976. The correct name for the type species of *Clavulinopsis*. Taxon 25: 515-516.

Petersen RH. 1978a. Notes on clavarioid fungi. XV. Reorganization of *Clavaria*, *Clavulinopsis* and *Ramariopsis*. Mycologia 70: 660-671.

Petersen RH. 1978b. The genus *Ramariopsis* in South-eastern Australia. Aus. J. Bot. 26: 425-431.

Petersen RH. 1978c. The genus *Clavaria* in South-eastern Australia. Aus. J. Bot. 26: 415-424.

Petersen RH. 1978d. Type studies in clavarioid fungi. V. A few Australian taxa. Mycotaxon 7: 386-392.

Petersen RH. 1979. Notes in clavarioid fungi XVII. *Clavulinopsis* taxa in south-eastern Australia. Sydowia 32: 209-223.

Petersen RH. 1980a. Type studies in the *Clavariaceae*. VI. Four pivotal types from the Pacific tropics. Mycotaxon 12: 281-216.

Petersen RH. 1980b. Type studies in clavarioid fungi. VI. The taxa described by Caspar van Overeem. Reinwardtia 9: 415-420.

Petersen RH. 1984. Type studies in the clavarioid fungi VIII. Persoonia 12: 225-237.

Petersen RH. 1985. Notes on clavarioid fungi. XIX. Colored illustrations of selected taxa, with comments on *Cantharellus*. Nova Hedwigia 42: 151-160.

Petersen RH. 1988a. The clavarioid fungi of New Zealand. DSIR Science Information Publishing Centre, Wellington.

Petersen RH. 1988b. Notes on clavarioid fungi. XXII. Three interesting South American collections. Mycologia 80: 571-576.

Petersen RH. 1989. Some clavarioid fungi from Northern China. Mycosistema 2: 159-173.

Petersen RH, Olexia PO. 1967. Type studies in the clavarioid fungi I. The taxa described by Charles Horton Peck. Mycologia 59: 767-802.

Petersen RH, Olexia PD. 1969. Notes on clavarioid fungi. XI. Miscellaneous notes on *Clavaria*. Can. J. Bot. 47: 1133-1142.

Pilát A. 1971. Species nova turficola generis *Ramariopsis* (Donk) Corner: *Ramariopsis subartica* sp. nov. Ceska Mycol. 25: 10-11.

Pine E, Hibbett DS, Donoghue MJ. 1999. Phylogenetic relationships of canthareloid and clavarioid Homobasidiomycetes based on mitochondrial and nuclear rDNA sequences. Mycologia 91: 944-963.

Poe S, Wiens JJ. 2000. Character selection and the methodology of morphological phylogenetics.

In Wiens JJ (ed.). Phylogenetic analysis of morphological data. Smithsonian Institution Press, Washington.

Restivo JH, Petersen RH. 1976. Studies on nuclear division and behavior within basidial I. *Hydnum umbilicatum*. Mycologia 68: 666-672.

Rieppel O. 1988. Fundamentals of comparative biology. Birkhäuser Verlag, Basel.

Rieppel O, Kearney M. 2002. Similarity. Biol. J. Linnean Soc. 75: 59-82.

Roth L. 1988. The biological basis of homology. In Humphries CJ (ed.). Ontogeny and systematics. Columbia University Press, New York.

Schild E. 1971. Fungorum rariorum icones colorate. Pars V. Clavariales. J. Cramer, Lehre.

Schild E. 1972. Ein seltener Korallenpilz. Z. Pilzk. 38: 23-29.

Singer R. 1945. New genera of fungi-II. Lloydia 8: 138-144.

Singer R. 1986. The Agaricales in modern taxonomy, 4th edition. Koeltz Scientific Books, Koenigstein.

Steglich W, Steffan B, Stroech K, Wolf M. 1984. Pistillarin, ein charakteristischer Inhaltsstoff der Herkuleskeule (*Clavariadelphus pistillaris*) und einiger *Ramaria*–Arten (Basidiomycetes). Zeit. Natur. 39c: 10-12.

Swofford DL. 2002. PAUP*. Phylogenetic analysis using parsimony (*and other metods), version 4.0b10. Sinauer.

Thind KS. 1961. The *Clavariaceae* of India. Indian Council of Agricultural Research, New Delhi.

Templeton AR. 1983. Phylogenetic inference from restriction endonuclease cleavage site maps with particular reference to the evolution of humans and apes. Evolution 37: 221-244.

Tyler VEJ. 1971. Chemiotaxonomy in the Basidiomycetes. In Petersen RH (ed.). Evolution in the higher Basidiomycetes. The University of Tennessee Press, Knoxville: 29-58.

Villegas M, De Luna E, Cifuentes J, Estrada-Torres E. 1999. Phylogenetic studies in *Gomphaceae* sensu lato (Fungi: Basidiomycetes). Mycotaxon 70: 127-147.

Wheeler QD. 2004. Taxonomic triage and the poverty of phylogeny. Phil. Trans. R. Soc. Lond. B 359: 571-583.

Weinstein RN, Pfister DH, Iturriaga T. 2002. A phylogenetic study of the genus *Cookeina*. Mycologia 94: 673-682.

Wiens JJ. 2004. The role of morphological data in phylogeny reconstruction. Syst. Biol. 53: 653-661.

MYCOTAXON

Volume 94, pp. 293–301 October–December 2005

A dichotomous key to *Scutellospora* species (*Gigasporaceae*, Glomeromycota) using morphological characters

Gladstone A. Silva[1]; Leonor C. Maia[1*] & Sidney L. Stürmer[2]

*leonorcmaia@yahoo.com.br
gladstonesilva@yahoo.com
[1]Departamento de Micologia, CCB, UFPE
Av. Prof. Nelson Chaves, s/n, 50670-420, Recife, PE, Brazil

sturmer@furb.br
[2]Departamento de Ciências Naturais, FURB
Cx.P. 1507, 89010-971, Blumenau, SC, Brazil

Abstract—A key to the genus *Scutellospora* (Order *Glomerales*, Phylum *Glomeromycota*) is compiled based on the protologues of species descriptions and information on type cultures of INVAM website.

Key words—arbuscular mycorrhizal fungi, *Glomerales*, identification, spore wall

Introduction

The genus *Scutellospora* was separated from the genus *Gigaspora* by Walker & Sanders (1986) based on differences in germination characteristics, subcellular structure within spores, and morphology of extraradical auxiliary cells. Weight was assigned most heavily to germination properties, with germ tubes formed from the inner warty surface of the spore wall in *Gigaspora* and from a novel germination shield formed on flexible layers independent of the spore wall in *Scutellospora*. Etymology of *Scutellospora* affirms these criteria, as the name derives from *scutellum*, or small shield. The germination shield differentiates on a bi-layered flexible germinal wall unique to glomeromycotan fungi. When more than one of these germinal walls are present, the germination shield forms always on the innermost one. Cellular organization of spores consists of spore wall and one to three of these flexible germinal walls in *Scutellospora* while only the spore wall in *Gigaspora* species. Vegetative criteria for genus-level separation reside in auxiliary cells, which have a knobby surface in *Scutellospora* and distinctly echinulate surface in *Gigaspora*.

In their classification of arbuscular mycorrhizal fungi (AMF), Morton & Benny (1990) placed *Gigaspora* and *Scutellospora* within the family *Gigasporaceae*, suborder *Gigasporineae* and order *Glomerales*. Members of *Gigasporaceae* were grouped by densely staining arbuscules, knobby coiled hyphae, unique soil-borne auxiliary cells and formation of spores on unique sporogenous cells. *Gigasporaceae* currently is grouped in the order *Diversisporales* (Schußler et al. 2001) which consists of an eclectic mix of families with no discernible morphological evolutionary pattern as classified. Therefore, at the order level, the morphological evolution conflicts with the 18S rRNA gene sequence phylogeny. Patterns and processes in spore ontogenesis (Franke & Morton, 1994; Morton, 1995) provided useful insights into the origins and boundaries of spore subcellular characters for the family *Gigasporaceae*. These works provided the foundation for reinterpretations and resolution of species-level characters. Stürmer & Morton (1999) subsequently described a new species, *Scutellospora rubra*, based on spore ontogenetic data.

Species of *Scutellospora* are geographically widespread with a pandemic distribution. Species of this genus have been reported from North America (Koske, 1987) South America (Siqueira et al. 1989; Stürmer & Bellei 1994; Yano-Melo et al. 2003; Silva et al. 2005), Europe (Blaszkowski 1989), Africa (Ba et al. 1996), Asia (Saito & Varga 1991) and Australia (Koske 1975). Although they are commonly associated with hosts in sand dunes, (Koske & Walker 1985, 1986; Koske & Gemma 1996; Blaszkowski 1991), they also are found in other natural ecosystems such as West Java tropical rain forests (Kramadibrata et al. 2000), Venezuela sclerophyllous shrublands (Herrera-Peraza et al. 2001), USA tall grassland (Hetrick & Bloom 1983) and Germany alpine ecosystem (Blaschke 1991), Brazil savanna-like "cerrado" (Walker & Diederichs 1989) and "caatinga" vegetation (Souza et al. 2003) as well as in brazilian degraded areas (Yano-Melo et al. 2003; Silva et al. 2005). Nevertheless, species of *Scutellospora* have also been registered from diverse agroecosystems (Hetrick & Bloom, 1983; Miller et al., 1985; Siqueira et al., 1989; An et al., 1993).

Early keys for identification of arbuscular mycorrhizal fungi were developed by Hall & Fish (1979) and Trappe (1982). Koske & Walker (1985) provided a key for a subset of *Scutellospora* species with ornamented spore walls. With a substantial increase in the number of new species described and the robust morphological delineation amongst species, we deemed it an appropriate time to erect a more comprehensive dichotomous key to *Scutellospora*.

Material and Methods

Morphological characters of *Scutellospora* were obtained from species protologues and from descriptions of reference isolates in the International Culture Collection of Arbuscular Mycorrhizal Fungi (INVAM, West Virginia University, WV) webpages (http://invam.caf.wvu.edu/speciesID.htm). Nomenclature in species descriptions was revised and standardized according to that proposed by Morton et al. (1995). Of 32 described *Scutellospora* species, 25 were incorporated in the key. The seven unincorporated species were *S. alborosea, S. gilmorei, S. minuta, S. nigra, S. reticulata, S. savannicola* and *S. tricalypta*, all of which were described before any standardized terminology for spore structures was developed (Walker 1983). For the 25 species considered in the key, characters of 14 species were obtained from INVAM web pages and 11 from published species descriptions. Wherever possible, color designations (*e.g.*, 0/20/40/0) were based on the CMYK model (Cyan, Magenta, Yellow and Black) used as the basis for the INVAM color chart (http://invam.caf.wvu.edu/otherinfo/articles/colorchart.htm). Spelling of names follows recommendations of Walker & Trappe (1993).

Results

DICHOTOMOUS KEY TO SOME *SCUTELLOSPORA* SPECIES

1a. Spores with one germinal wall 2
1b. Spores with more than one germinal wall. 8

2a. (1a) Surface of spore wall smooth. 3
2b. Surface of spore wall ornamented 4

3a. (2a) Spores pale (0/0/5/0) to slightly darker cream (0/0/40/0) in color, with mean diameter of 180 μm (range 120-240 μm), second layer (L2) of the spore wall 4.5-6 μm thick (mean = 5.1 μm) . . . . ***S. fulgida***
3b. Spores chestnut (0/40/100/0) in color, with mean diameter of 300 μm, second layer (L2) of spore wall 10-25 μm thick . . . . ***S. castanea***

4a. (2b) Spore wall ornamented with warts 5
4b. Spore wall ornamented with blunt tapering projections 1-3 μm wide and 2 μm high, L2 of the spore wall swelling in acidic mountants and turning dark red-brown in Melzer's reagent .... ***S. biornata***

5a. (4a) Color of mature spore ranging from pale-straw (0/10/10/0) to orange-brown (0/60/100/0) or pale to dark copper (0/60/100/0 to 20/80/60/0). 6
5b. Color of mature spore darker than above, ranging from red-brown (20/80/100/10) to dark red -brown (40/80/80/0). 7

6a. (5a) Spores pale to dark copper to darker cream color (20/60/70/10), 240-360 μm in diameter (mean = 313 μm) with surface covered by rounded warts 0.5 μm wide and 0.2-0.5 μm high . . . . ***S. persica***
6b. Spores pale straw to orange brown, 220-360 μm in diameter (mean = 308 μm), with surface covered with tightly packed warts < 1 μm apart, 0.5-1.0 μm wide and 0.5-1.2 μm high . . . . ***S. verrucosa***

7a. (5b) Spore size of 260-480 μm (mean = 362 μm), spore surface covered with flattened warts with angular margins (2-12 μm wide and 1-3 μm high). . . . . . ***S. coralloidea***
7b. Spore size of 380-520 μm (mean = 473 μm), spore surface ornamented with rounded warts (11-26 μm wide and 3-7 μm high). . . . . ***S. gregaria***

8a. (1b) Spores with two germinal walls 9
8b. Spores with three germinal walls 24

9a. (8a) Surface of spore wall smooth. 10
9b. Surface of spore wall ornamented 18

10a. (9a) Spores pale to dark orange-brown (0/60/100/0) to red-brown (20/80/100/0) 11
10b. Spores lighter in color than above, color ranging from white/hyaline or pale yellow (5/0/20/0) to yellow (0/0/100/0) 12

11a. (10a) Spore size of 140-220 μm (mean = 180 μm). Second layer (L2) of the spore wall 3.5-12 μm thick staining dark red black (20/80/70/10) in Melzer's reagent. Second layer of germinal wall 2 is 0.6-1.4 μm thick staining pale pinkish purple (0/20/20/0) to darker pinkish purple (20/40/20/0) in Melzer's reagent . . ***S. rubra***

11b. Spore size of (200-) 240 (-360) x (180-) 230 (-290) μm (mean = 235 μm). Second layer (L2) of the spore wall 0.8 -2.2 μm not reacting in Melzer's reagent. Second layer of germinal wall 2 is 3 - 4 up to 25 μm thick, with amorphous characteristics, staining reddish purple (20/80/20/0) in Melzer's reagent . . . . ***S. hawaiiensis***

12a. (10b) Spore wall not reacting in Melzer's reagent 13

12b. Spore wall reacting in Melzer's reagent 15

13a. (12a) Spores pale yellow with a greenish tint (5/0/20/0). Spore size ranging from 120-240 μm. Second layer (L2) of spore wall finely laminated, ranging from 1.8-4.2 μm thick. Germinal wall 2 reacting in Melzer's reagent.. 14

13b. Spores pale yellow (5/0/20/0) to yellow (0/0/100/0) without a greenish tint. Spore size ranging from 320-440 x 320-470 μm. Second layer (L2) of spore wall coarsely laminated, ranging from 6 -16 μm thick. Germinal wall 2 not reacting in Melzer's reagent . . . . ***S. aurigloba***

14a. (13a) Spores with germinal wall 1 consisting of one hyaline layer 0.6-1.0 μm thick, staining light pink (0/20/20/0) in Melzer's reagent and often adherent to spore wall. Second layer (L2) of germinal wall 2 staining red-purple (20/80/20/0) to dark red-purple (40/80/60/0) .... ***S. dipurpurescens***

14b. Spores with germinal wall 1 consisting of two hyaline adherent layers that together are 0.9-2.0 μm thick. Second layer (L2) of germinal wall 2 staining red-purple (20/80/20/0) to dark red-purple (40/80/60/0) . . . . ***S. calospora***

15a. (12b) Spore color hyaline/white to pale pink (0/30/20/0) to deep pink (0/40/20/0) 16

15b. Spore color apricot yellow and yellow brown (0/5/40/0) to orange brown (0/60/100/0). 17

16a. (15a) Spore color pale pink (0/30/20/0) to deep pink (0/40/20/0), turning hyaline when the pink color fades. Spore wall color turns yellowish with a greenish tint (0/0/80/). Second layer of germinal wall 2 turns pale pink (0/20/20/0) to light purple (20/40/20/0) in Melzer's reagent. . . . . ***S. weresubiae***

16b. Spore color hyaline/white. Spore wall: first layer (L1), hyaline, 1.8-5.0 μm thick and second layer (L2) 3.0-8.8 μm thick. Spore wall turns dark red-purple (40/80/40/0) to reddish-black (60/80/50/10) in Melzer's reagent. Second layer (L2) of germinal wall 2 with characteristics "amorphous" staining red purple (20/80/20/0) to dark red purple (40/80/60/0) in Melzer's reagent . . . . ***S. pellucida***

17a. (15b) Spore color apricot yellow to yellow brown (0/5/40/0). Spore wall turning garnet red in Melzer's reagent. Second layer (L2) of germinal wall 2 with characteristics "amorphous" staining red purple (20/80/20/0) in Melzer's reagent . . . . ***S. armeniaca***

17b. Spore color pale yellow-brown (0/10/20/0 to 0/10/60/0) to orange brown (0/60/100/0). Spore wall turning dark red-brown in Melzer's reagent. Second layer (L2) of germinal wall 2 with characteristics "amorphous" staining dark reddish purple (40/80/40/0) in Melzer's reagent... .... ***S. arenicola***

18a. (9b) Spore color pale orange brown (0/60/80/0) and dark orange brown (0/60/100/10) to red brown (40/80/100/0) 19

18b. Spore color lighter than above, ranging from hyaline/white, pale cream (0/10/40/0) to metallic gold (0/20/100/0) 20

19a. (18a) Spore surface ornamented with short rounded warts 1.0-2.5 μm high. Second layer (L2) of spore wall 5-9 μm thick (mean = 7.4 μm), staining dark red brown (20/80/70/10) in Melzer's reagent........................... ***S. heterogama***

19b. Spore surface with two types of ornamentation: crowded small conical warts, 0.5-1 μm wide and 0.5-1.5 μm high and hyaline, blunt, bacilliform large projections 1.5-3 μm wide and 2-10 μm high. Second layer (L2) of spore wall 3-8 μm thick. Reaction of spore wall to Melzer's reagent unknown ....***S. dipapillosa***

20a. (18b) Spore wall reacting on Melzer's reagent turning dark red-purple (40/80/40/0) to reddish-black (60/80/50/10) 21

20b. Not as above 22

21a. (20a) Spore size of 220-380 μm (mean = 300 μm). Spore wall ornamented with papilla rarely > 1 μm high and 1-1.5 μm wide. Second layer (L2) of germinal wall 2 with characteristics of "amorphous" and stains red purple (20/80/20/0) to dark red purple (40/80/60/0) in Melzer's reagent ....***S. cerradensis***

21b. Spore size of 160-270 μm (mean = 240 μm). Spore wall ornamented with knobs 3.5 6.5μm high and 7-10.5 μm wide. Second layer (L2) of germinal wall 2 with characteristics of "amorphous" and stains dark red purple (40/80/60/0) in Melzer's reagent .... ***S. nodosa***

22a. (20b) Ornamentation of the spore wall 2-4 μm high 23

22b. Ornamentation of the spore wall 3-6 μm heigh and 3.6-10 μm wide consisting of dome-like sub-polygonal papillae. Spore wall not swelling in acidic mountants. L2 of the germinal wall 2 turning red purple (20/80/20/0) in Melzer's reagent .... ***S. crenulata***

23a. (22a) Spore ornamented with columnar protuberances 2-4 μm long. Second layer (L2) of germinal wall 2 staining purple (40/60/20/0) in Melzer's reagent ...... ***S. projecturata***

23b. Spore ornamented with dense blunt spines 2-4 μm long. Second layer (L2) of germinal wall 2 staining red purple (20/80/20/0) in Melzer's reagent........... ***S. spinosissima***

24a. (8b) Spores hyaline/white to yellow brown (0/5/40/0) in older spores, 340-640 μm in diameter (mean = 495 μm), with dark yellow-brown germination shield highly contrasting with spore color. Spore wall not reacting in Melzer's reagent. Second layer (L2) of germinal wall 3 staining red purple (20/80/20/0) to dark red-purple (40/80/60/0) in Melzer's reagent... ....*S. scutata*

24b. Spores red-brown (20/80/60/0) to dark red-brown (20/80/100/10), subglobose to oblong, 160-320 μm diameter (mean = 240 μm). Spore wall not reacting in Melzer's reagent. Second layer (L2) of germinal wall 3 staining pinkish (0/40/20/0) to pinkish purple (0/60/30/10) ....*S. erythropa*

Discussion

This paper provides the first dichotomous key of *Scutellospora* since Koske & Walker's key of approximately two decades ago for species with roughened (ornamented) spore walls. The first character in the key is number of germinal walls present in the spore, which can be readily detected by both beginning and experienced researchers once they recognize the fundamental bi-layered organization of each wall. From there, properties of spore wall layers (color, presence and type of ornamentation, etc.) discriminate amongst species (Morton et al. 1995).

Spore wall structure was re-interpreted from published species descriptions of some species according to developmental patterns (Morton et al. 1995). For example, Koske & Walker (1985) described *S. coralloidea, S. gregaria, S. verrucosa* and *S. persica* as possessing a hyaline, membranous wall. Developmental patterns indicate this structure represents one germinal wall composed by two layers (Morton 1995). Based on this type of comparison, *S. hawaiiensis, S. aurigloba, S. weresubiae, S. dipapillosa, S. nodosa, S. projecturata, S. spinosissima*, *S. arenicola* and *S. crenulata* were considered to have two germinal walls. To support this interpretation, Melzer's reaction of germinal walls was also considered. *Scutellospora* species with only one germinal wall shows no reaction in Melzer's (Morton, 1995) while those with two or three germinal walls have the second layer of the innermost wall turning light to dark purple in Melzer's reagent. For instance, protologues of *S. arenicola* and *S. crenulata* are not straightforward about the number of germinal walls, however, both become red purple in Melzer's reagent; we therefore interpreted these species as having two germinal walls. Establishment of these organisms in single-species culture will help to clarify this interpretation.

The presence of one, two or three germinal walls defines groups of species within *Scutellospora*. Within each group, new subgroupings emerge when considering characteristics of spore wall and germinal wall. Therefore, within *Scutellospora* species with one germinal wall, two subgroups are formed relative to ornamentation of spore wall. One group is formed by *S. fulgida* and *S. castanea*, both of which have smooth spore walls, whereas another group is formed by *S. biornata, S. persica, S. verrucosa, S. coralloidea* and *S. gregaria* which produce a spore wall ornamented with blunt tapering projection and warts. We are not suggesting that they represent new taxa (*e.g.*, subgenus), but this grouping is very useful during species identification.

Within *Scutellospora* species with two germinal walls, subgroupings based on spore color and ornamentation also emerge. Therefore, there are species producing smooth spore walls with dark spore color (*e.g.*, *S. rubra* and *S. hawaiiensis*) and those with pale spore color (*e.g.*, *S. aurigloba, S. dipurpurescens, S. calospora, S. weresubiae, S. pellucida*, *S. armeniaca* and *S. arenicola*). The third subgroup includes species producing ornamented spores (*S. heterogama, S. dipapillosa, S. cerradensis, S. nodosa, S. crenulata, S. projecturata* and *S. spinosissima*). The third large group within the genus includes species differentiating three germinal walls like *S. scutata* and *S. erythropa*, which can be readily separated from each other based on spore color and size (hyaline/white larger spores in the former and red-brown smaller spores in the latter).

Differences among some species now described in the genus *Scutellospora* can be small so that species boundaries are not clear. For example, the main difference between *S. dipurpurescens* and *S. calospora* is the presence of one or two layers in the first germinal wall, respectively. Spore size, color and Melzer's reaction of germinal wall 2 are identical between these two species. On the same token, differences between *S. heterogama* and *S. dipapillosa* reside mostly on the very small type of ornamentation occurring on the latter, a characteristic already noticed by Koske & Walker (1985). Some spores of *S. dipapillosa* are identical to *S. heterogama*, while others that show the variation described by Koske & Walker (1985) are present in parasitized spores – so the difference may be an artifact of parasitism rather than a heritable difference (J.B. Morton, personal communication). *Scutellospora pellucida* and *S. armeniaca* are also very similar and may represent the same species; both have the same range of spore size and Melzer's reaction of spore wall and germinal wall 2 are identical. Spore color can be used with caution to separate both species, as *S. armeniaca* produces spores apricot yellow and yellow brown while *S. pellucida* are hyaline. However, spores from the latter can be pale yellow brown when older or extracted from roots in pot cultures or from the field, the condition where spores from the former were described (J.B. Morton, personal communication).

We proposed this dichotomous key to help mycorrhizologists to identify *Scutellospora* species during field surveys or for establishing isolates in single-species cultures. We are aware that interpretation of spore wall structure for some species might not be adequate, especially those not established in monospecific culture. Therefore, we suggest that for further species description, spore wall characteristics should be described using nomenclature based on spore developmental model as proposed by Morton et al. (1995).

Acknowledgments

This work is part of the thesis of the first author, who received a scholarship from CAPES/Brazil to obtain the degree of Doctor in Mycology. Leonor C. Maia acknowledges the support from CNPq/Brazil. We are grateful to Dr. Joseph B. Morton (West Virginia University, WV) and Dr. Stephen P. Bentivenga (University of Oshkosh, WI) for helpful pre-submission reviews.

Literature Cited

An ZQ, Guo BZ, Hendrix JW. 1993. Populations of spores and propagules of mycorrhizal fungi in relation to the life cycles of tall fescue and tobacco. Soil Biol. Biochem. 25: 813-817.

Ba AM, Dalpe Y, Guissou T. 1996. Les *Glomales* d'*Acacia holosericea* at d'*Acacia mangium.* Bois et Forets des Tropiques (250): 5-18.

Bentivenga SP, Morton JB. 1995. A monograph of the genus *Gigaspora*, incorporating developmental patterns of morphological characters. *Mycologia* 87: 720-732.

Blaschke H. 1991. Distribution, mycorrhizal infection, and structure of roots of calcicole floral elements at treeline, Bavarian Alps, Germany. Arctic Alp. Res. 23: 444-450.

Blaszkowski J. 1989. The occurrence of the *Endogonaceae* in Poland. Agriculture, Ecosystems and Environment 29: 45-50.

Blaszkowski J. 1991. Polish Glomales VIII. *Scutellospora nodosa*, a new species with knobby spores. Mycologia 83: 537-542.

Franke M, Morton JB. 1994. Ontogenetic comparisons of arbuscular mycorrhizal fungi *Scutellospora heterogama* and *Scutellospora pellucida*: revision of taxonomic character concepts, species descriptions, and phylogenetic hypotheses. Can. J. Bot. 72: 122-134.

Hall IR, Fish BJ. 1979. A key to the *Endogonaceae.* Trans. Br. Mycol. Soc. 73: 261-270.

Herrera-Peraza RA, Cuenca G, Walker C. 2001. *Scutellospora crenulata,* a new species of *Glomale*s from La Gran Sabana, Venezuela. Can. J. Bot. 79: 674-678.

Hetrick BAD, Bloom J. 1983. Vesicular-arbuscular mycorrhizal fungi associated with native tall grass prairie and cultivated winter wheat. Can. J. Bot. 61: 2140-2146.

Miller DD, Domoto PA, Walker C. 1985. Mycorrhizal fungi at eighteen apple rootstock plantings in the United States. New Phytol. 100: 379-391.

Morton JB, Benny GL. 1990. Revised classifiation of arbuscular mycorrhizal fungi (Zygomycetes): a new Order, *Glomales*, two new Suborders, *Glomineae* and *Gigasporineae*, and two new Families, *Acaulosporaceae* and *Gigasporaceae*, with an emendation of *Glomaceae*. Mycotaxon 37: 471-491.

Morton JB. 1995. Taxonomic and phylogenetic divergence among five *Scutellospora* species based on comparative developmental sequences. Mycologia 87: 127-137.

Morton JB, Bentivenga SP, Bever JD. 1995. Discovery, measurement, and interpretation of diversity in arbuscular endomycorrhizal fungi (*Glomales*, Zygomycetes). Can. J. Bot. 73: 25-32.

Koske RE. 1975. *Endogone* spores in Australian sand dunes. Can. J. Bot. 53: 668-672.

Koske RE. 1987. Distribution of VA mycorrhizal fungi along a latitudinal temperature gradient. Mycologia 79: 55-68.

Koske RE, Walker C. 1985. Species of *Gigaspora* (*Endogonaceae*) with roughned outer walls. Mycologia 77: 702-720.

Koske RE, Walker C. 1986. Species of *Scutellospora* (*Endogonaceae*) with smooth-walled spores from maritime sand dunes: two new species and a redescription of the spores of *Scutellospora pellucida* and *Scutellospora calospora*. Mycotaxon 27: 219-235.

Koske RE, Halvorson WL. 1989. *Scutellospora arenicola* and *Glomus trimurales:* two new species in the *Endogonaceae*. Mycologia 81: 927-933.

Koske RE, Gemma JN. 1996. Arbuscular mycorrhizal fungi in Hawaiian sand dunes: Island of Kauai. Pacific Sci. 50: 36-45.

Kramadibrata K, Walker C, Schwarzott D, Schüßler A. 2000. A new species of *Scutellospora* with a coiled germination shield. Ann. Bot. 86: 21-27.

Saito M, Vargas R. 1991. Vesicular-arbuscular mycorrhizal fungi in some humus-rich Ando soils of Japan. Soil Microorg. 38: 3-15.

Schußler A., Schwarzott D, Walker C. 2001. A new fungal phylum, the Glomeromycota: phylogeny and evolution. Mycol. Res. 105: 1413-1421.

Siqueira JO, Colozzi-Filho A, Oliveira E. 1989. Ocorrência de micorrizas vesicular-arbusculares em agro e ecossistemas do estado de Minas Gerais. Pesq. Agropec. Bras. 24: 1499-1506.

Silva GA, Trufem SFB, Saggin-Júnior OF, Maia LC. 2005. Arbuscular mycorrhizal fungi in a semiarid copper mining area in Brazil. Mycorrhiza 15: 47-53.

Souza RG, Maia LC, Sales MF, Trufem SFB. 2003. Diversidade e potencial de infectividade de fungos micorrízicos arbusculares em área de caatinga, na Região de Xingó, Estado de Alagoas, Brasil. Rev. Bras. Bot. 26: 49-60.

Stürmer SL, Bellei MM. 1994. Composition and seasonal variation of spore populations of arbuscular mycorrhizal fungi in dune soils on the Island of Santa Catarina, Brazil. Can. J. Bot. 72: 359-363.

Stürmer SL, Morton JB. 1999. *Scutellospora rubra*, a new arbuscular mycorrhizal species from Brazil. Mycol. Res. 103: 949-954.

Trappe JM. 1982. Synoptic key to the genera and species of Zygomycetous mycorrhizal fungi. Phytopathology 72: 1102-1108.

Walker C. 1983. Taxonomic concepts in the *Endogonaceae*: spore wall characteristics in species descriptions. Mycotaxon 18: 443-455.

Walker C, Sanders FE. 1986. Taxonomic concepts in the *Endogonaceae*: III. The separation of *Scutellospora* gen. nov. from *Gigaspora* Gerd. & Trappe. Mycotaxon 27: 169-182.

Walker C, Diederichs C. 1989. *Scutellospora scutata* sp. nov., a newly described endomycorrhizal fungus from Brazil. Mycotaxon 35: 357-361.

Walker C, Trappe JM. 1993. Names and epithets in the *Glomales* and *Endogonales*. Mycol. Res. 97: 339-344.

Yano-Melo AM, Trufem SFB, Maia LC. 2003. Arbuscular mycorrhizal fungi in salinized and surrounded areas at the São Francisco Submedium Valley, Brazil. Hoehnea 30: 79-87.

MYCOTAXON

Volume 94, pp. 303–324 October–December 2005

New species and phylogenetic relationships of *Hypoxylon* species found in Thailand inferred from the internal transcribed spacer regions of ribosomal DNA sequences

N. Suwannasai[1*], S. Rodtong[1],
S. Thienhirun[2] & A. J. S. Whalley[3]

*snuttika@hotmail.com
[1]School of Microbiology, Institute of Science, Suranaree University of Technology
Nakhon Ratchasima 30000 Thailand

[2]Forest Management and Forest Products Research Office Royal Forest Department
Chatuchak, Bangkok 10900 Thailand

[3]School of Biomolecular Sciences, Liverpool John Moores University
Liverpool L3 3AF U.K.

Abstract—*Hypoxylon* specimens collected from different forest areas in Thailand were investigated and identified to species using both morphological and molecular characteristics. Fifteen species, including three new species, *H. kanchanapisekii*, *H. sublenormandii*, and *H. suranareei*, were recorded. The phylogenetic tree of the *Hypoxylon* examined was constructed using the neighbour-joining method based on the internal transcribed spacer (ITS) regions including 5.8S ribosomal DNA sequences. The molecular results revealed clear separation among *Hypoxylon* species including closely related species or on data based only on teleomorphic DNA. Three taxa in *Hypoxylon* sect. *Annulata*—*H. nitens*, *H. purpureonitens*, and *H. bovei* var. *microspora*—were grouped together, but the fourth species, *H.* cf. *archeri*, was separated. The phylogenetic tree does not support the monophyletic group of sect. *Annulata* in this study. In addition, these molecular data could be used to confirm the recognition of new species based on morphological features and they are valuable for the creation of the *Hypoxylon* DNA sequence database.

Key words—*Xylariaceae*, *Annulohypoxylon*, taxonomy

Introduction

Hypoxylon Bull. is a large complex genus of the family *Xylariaceae* which is a well known family of the Ascomycota (Whalley 1996, Rogers 2000). They are distributed worldwide, being especially common in subtropical and tropical regions. Their habitat is on wood. Most *Hypoxylon* species are saprotrophs involved in the decay of wood or occasionally being weak parasites in stressed host trees (Whalley1996, Edwards et al. 2003). Moreover an increasing number of *Hypoxylon* species being reported as endophytes in a wide range of living plants (Rodrigues & Petrini 1997; Rogers 2000; Edwards et al. 2003).

Traditionally, *Hypoxylon* has been characterized by morphological characteristics based on stromatal structure, pigmentation, shape and features of the ascospore (Miller 1961; Martin 1968; Ju & Rogers 1996). Thus, the delimitation of the genus *Hypoxylon* has been the subject of several rearrangements since the monograph of Miller (Miller 1961). Miller's monograph divided the genus into four sections, *Hypoxylon*, *Annulata*, *Applanata* and *Papillata* strongly relying on stromatal form, texture, colour, and ostiole form (Miller 1961). Consequently, this monograph failed to recognize the relationships among species and groups of species. The section *Applanata* sensu Miller has since been redistributed between *Camillea* Fr. and *Biscogniauxia* Kuntze (Læssøe, Rogers & Whalley 1989; Whalley, Læssøe & Kile 1990; Ju et al. 1998) whereas members of the section *Papillata* subsection *Primocinerea* (Miller 1961) have been allocated to a range of genera including *Nemania* Gray emend. Pouzar (Pouzar 1985), *Rosellinia* De Not. (Petrini 1992), *Kretzschmaria* Fr. (Van der Gucht 1994), *Kretzschmariella* Viégas (Ju & Rogers 1994), *Entoleuca* Syd. (Rogers & Ju 1996), and *Euepixylon* Füisting (Læssøe & Spooner 1994). Recently, *Hypoxylon* was revised by Ju & Rogers (1996) using four major criteria to define the genus: *Nodulisporium*-like anamorphs, stromata unipartite, never erect, with a solid and homogenous basal tissue below the perithecial layer. They divided *Hypoxylon* into sections *Hypoxylon* and *Annulata* separating them on the basis of presence or absence of a layer of carbonaceous stromatal tissue enclosing perithecia. Additional major characters used to separate the two sections included the perispore, which if dehiscent, exhibits a visible thickened area positioned about one-third of the length of the ascospore and on the same side of the germination slit in members of the Annulata, and the presence or absence of an annular disc surrounding the ostiolum. Ju & Rogers (1996) accepted at least 130 species and varieties and have since recognized a further 16 species and one variety (Ju Rogers & Hsieh 2004). When revising the genus Ju & Rogers (1996) were able to utilize data absent from the monograph of Miller (1961), such as ascospore ornamentation using scanning electron microscopy, form of the apical apparatus of the ascus, germination slit morphology, and the colour of stromatal pigments extracted with 10% potassium hydroxide (KOH) solution. However, in spite of their revision identification of certain *Hypoxylon* species remains problematic with insufficient discriminatory morphological information to clearly delimit certain taxa and for the confident identification of individual species. This has proved to be most pronounced when considering tropical taxa of the *Annulata* especially *Hypoxylon nitens* (Ces.) Y.-M. Ju & J.D. Rogers, *H. moriforme* Henn., *H. bovei* Speg. var. *microspora* J.H. Miller and *H. purpureonitens* Y.-M. Ju & J.D. Rogers where, between them, there is considerable variation and overlap in their morphological features.

Molecular studies based on DNA sequences are recognized as more reliable methods to reveal genetic relationships, and can be used to evaluate the relationships of fungi at any taxonomic rank (Bruns et al. 1991). Fungal ribosomal DNA (rDNA) genes are frequently used because these genes contain highly conserved and variable regions, and they are found in multiple copies per genome (Gardes & Bruns 1993). In the rDNA genes, the regions of the internal transcribed spacers (ITS) 1 and 2 including 5.8S ribosomal DNA gene have been extensively studied to differentiate taxonomic entities at the species level in several fungal genera, such as *Fusarium* Link (O'Donnell 1992), *Penicillium* Link (Skouboe et al. 1999), *Xylaria* Hill *ex* Schrank (Lee et al. 2000), and *Hypoxylon* (Sánchez-Ballesteros et al. 2000, Mazzaglia et al. 2001). Sequence data of rDNA provides a powerful tool for evaluating phylogenetic relationship among different taxa especially at higher taxonomic levels. Moreover DNA sequence data from ITS1-5.8S-ITS2 region has been used to develop specific oligonucleotide primers of pathogenic fungi such as *Penicillium marneffei* Segr., Capp. & Sur. (LoBuglio & Taylor 1995).

In this study we elected to investigate the genetic variation within *Hypoxylon* species in order to obtain correlations between their morphological and molecular characteristics using DNA sequences of the ITS1-5.8S-ITS2 region. It is our belief that the application of these data in conjunction with morphological features will provide a better definition of species delimitation of *Hypoxylon*. Since our study was completed Hsieh et al. (2005) have published on molecular phylogeny of *Hypoxylon* and closely related genera based on β-tubulin and α-actin gene sequences. They proposed a new genus *Annulohypoxylon* Y.-M. Ju, J.D. Rogers & H.-M. Hsieh to accommodate taxa originally assigned to *Hypoxylon* sect. *Annulata* (Ju & Rogers 1996).

Materials and Methods

Fungal materials

Eighty-nine collections of *Hypoxylon* specimens were collected from different forest areas in Thailand. They were classified and identified to species level using morphological characters (Ju & Rogers 1996; Ju Rogers & Hsieh 2004), and cultures were obtained whenever possible following ascospore discharge, germination and colony development. The stromatal pigments of *Hypoxylon* were extracted by using 10% potassium hydroxide (KOH) solution, left for one minute, and observed the colour compared to the colour chart of Rayner (1970). Nine *Hypoxylon* isolates were additionally obtained from the Royal Forest Department (Thailand). Two species (as teleomorphs) of *H. nitens* and *H. bovei* var. *microspora* were provided by Dr. Yu-Ming Ju (National University of Taiwan, Taiwan). Details of the representative cultures of the *Hypoxylon* species investigated together with the two Taiwanese collections are listed in Table 1. All isolates were routinely grown on potato dextrose agar at 25°C, and maintained in 15% glycerol at -20°C.

DNA extraction, ITS1-5.8S-ITS2 region amplification and DNA sequencing

Genomic DNA was extracted from cultural mycelia and from stromatal herbarium materials by using the method of Lee & Taylor (1990) with some modifications. The internal transcribed spacer (ITS) regions 1 and 2 including 5.8S ribosomal nucleotide sequence were amplified by polymerase chain reaction (PCR) with the fungal specific primers ITS4 (5'-TCCTCCGCTTCTTGATAGC-3') and ITS5 (5'-GGAAGTAAAAGTCGTAACAAGG-3') (White et al. 1990). The PCR reaction was carried out in 50 μl mixtures containing 10 ng of DNA, 200 μM of each dNTP (dATP, dGTP, dCTP, and dTTP), 2.5 μM of each primer, 2 mM of $MgCl_2$, and 1 unit of *Taq* Polymerase (Sigma, U.S.A). The PCR cycle of ITS amplification consisted of 1 cycle of 95°C for 5 min; 35 cycles of 95°C for 30 sec, 53°C for 30 sec, 72°C for 1 min; and the final cycle of 72°C for 10 min. PCR reactions were carried out in an automated thermal cycler (BioRad, U.S.A).

Amplification products were purified through columns using the QIA quick PCR Purification kit (Qiagen, U.S.A) and sequenced using the PCR primers as sequencing primers with the Big Dye DNA Sequencing kit (Applied Biosystems, U.S.A). All reactions were then run on a Perkin Elmer ABI PRISM^{TM} 377 DNA Sequencer (Applied Biosystems, U.S.A).

Phylogenetic analysis

All DNA sequences of rDNA were determined and trimmed to remove all 18S and 28S sequences according to sequence boundaries defined by Sánchez-Ballesteros et al. (2000). DNA sequences of ITS regions were analyzed and aligned manually using ClustalW (Thompson et al. 1994) and Bioedit program version 4.7.1 created by Tom Hall (Department of Microbiology, North Carolina State University, Raleigh, U.S.A). Genetic distances for the neighbour-joining and conditional clustering, Kimura 2 parameter distances (Kimura 1980), were computed with the Dnadist module of the PHYLIP software package (Felsenstein 1995). Selected ITS sequences of related species in the GenBank database (http://www.ncbi.nlm.nih.org) were included for comparison with sequences obtained in the current study. Strengths of internal branches of resulting trees were statistically tested by the bootstrap analysis of 1000 replications.

Results

Fungal collection and identification

Eighty nine *Hypoxylon* collections were classified and identified as belonging to 15 species including 3 unique new species, on the basis of their morphological characteristics (Table 1). Details of the new species are given below under taxonomic descriptions. The collections exhibited high variation in their morphological characteristics, e.g. stromatal surface colour, ascospore size and shape, and germination slit morphology. The morphological characteristics of representatives of each species recognized are presented in Table 2.

Taxonomic Descriptions

Morphological features of the Thai collections identified as *H. anthochroum*, *H. bovei* var. *microspora*, *H. fendleri*, *H. haematostroma*, *H. lenormandii* (except for two collections on bamboo), *H. monticulosum*, *H. nitens*, *H. purpureonitens*, *H. stygium*, *H. atroroseum* and *H. rubiginosum* were all in very close agreement with the descriptions for those species (Ju & Rogers 1996) or with the characters of voucher specimens provided by Dr. Yu-Ming Ju. Four collections initially considered being close to *H. archeri* grouped together in clade III but were separated from the other annulate species suggesting that they represent the same taxon. The presence of a white fringe surrounding their ostioles and other minor deviations from typical *H. archeri* suggest that they all represent a new taxon. Three collections from Ratchaburi Province are described as *H. kanchanapisekii* named to honour the Royal Golden Jubilee Ph.D. Program, which provides the financial support for this study. Two collections similar to *H. lenormandii* but growing on bamboo are described as a new species named *H. sublenormandii*. Another new species, *H. suranareei*, is named following the location of specimen collections at Suranaree University of Technology, Thailand.

Hypoxylon kanchanapisekii Suwannasai, Rodtong, Thienhirun & Whalley, **sp. nov.**

FIGURES **1** A-F

> *Stromata glomerata vel pulvinata, 0.5-2mm diam. X 0.1-0.2mm crassa; tumulis peritheciorum inconspicuis vel conspicuis, externe obscure rubro-brunnea vel umbrinis in KOH dissolutis; Perithecia globosa 0.1-0.2 mm diam. Ostiola plana vel parum elevata. Asci 105-120 µm longitudine tota x 3.8-5 µm crassa, partibus sporiferis 75-85 µm longitudine stipitibus 12.5-35 µm longitudine annulo apicali in liquore iodato Melzeri cyanescente, discoideo, 1.25 µm alto x 2.5 µm lato. Ascosporae brunneae, unicellulares, ellipsoideae cum apicibus angustatis, 10-11.5(-12.5) x (1-)3.5-5 µm, rima germinativa recta longa praeditae; perisporium in KOH indehiscens, leve; episporium leve.*

KEY CHARACTERS: **Stromata** glomerate to pulvinate, restricted and usually containing less than 20 perithecia, perithecia occasionally almost free, 0.5-2 mm x 0.1-0.2 mm thick, with perithecial mounds inconspicuous to 1/3 exposed, surface dull reddish brown with KOH extractable pigments brown vinaceous (84), or umber (9); **perithecia** spherical 0.1-0.2 mm diam.; **ostioles** slightly higher or the same as the stromatal surface; **asci** 105-120 µm total length x 3.8-5 µm broad, the spore bearing parts 75-85 µm long with stipes 12.5-35 µm; **ascospores** brown, unicellular, equilateral, with narrowly rounded ends, 10-11.5(-12.5) x (1-)3.5-5 µm, with straight 2/3 length germ-slit; **perispore** indehiscent in 10% KOH, smooth, **epispore** smooth; **colonies** on PDA covering 9-cm Petri dish in two weeks at room temperature, 23-28°C, at first cream then buff, velvety to felty, with concentric zones where aerial hyphal tufts develop; **anamorph** not formed.

> **SPECIMENS EXAMINED:** THAILAND, Ratchaburi Province, the Royal Forest Department, on bamboo, 28 August 2003, Suwannasai, N. (Holotype *SUT069*); *SUT066*; *SUT068*.

Comments: *Hypoxylon kanchanapisekii* is close to *H. parksianum* Y.-M. Ju & J.D. Rogers (Ju & Rogers 1996) except that the stromatal form of *H. kanchanapisekii* is normally glomerate and contains less than 20 perithecia whereas *H. parksianum* is pulvinate to effused-pulvinate with inconspicuous to conspicuous perithecial mounds. The colour of granules, which beneath surface and between perithecia, of *H. kanchanapisekii* is dull reddish brown but it is blackish in *H. parksianum*. The perithecia of *H. kanchanapisekii* are spherical with 0.1-0.2 mm in diameter, whereas *H. parksianum* perithecia are spherical to tubular and larger size, 0.3-0.6 mm in diameter x 0.4-0.8 mm high. Asci of *H. parksianum* are longer, 147-174 µm total length x 6.5-7.5 µm broad, than *H. kanchanapisekii*, which are described above. Ascospores of *H. kanchanapisekii* are slightly smaller than *H. parksianum* (11-14.5 x (4.5-) 5-6 µm). Host of this new species is known only from bamboo clump while the substrate of *H. parksianum* is on corticated wood of *Hibiscus*.

Hypoxylon sublenormandii Suwannasai, Rodtong, Thienhirun & Whalley, **sp. nov.**

FIGURES **2** A-D

> *A Hypoxylo lenormandii differt in ascosporis 8.9-11.3 x 3.4-4.7 µm et in rima germinativa recta longa praeditae.*

KEY CHARACTERS: **Stromata** glomerate to effused-pulvinate, often appearing almost rosellinioid but joined by thin stromal tissue, conspicuous perithecial mounds, surface reddish brown, reddish brown granules immediately beneath surface and between perithecia, with KOH-extractable pigments brown vinaceous (84), or umber (9); **perithecia** spherical, 0.2-0.4 mm diam., ostioles slightly higher than the stromatal surface; **asci** 95-110 µm total length x 3.8-5 µm broad, the spore bearing parts 65-75 µm long with stipes 30-42.5 µm; **ascospores** brown, unicellular, ellipsoid-inequilateral, with narrowly rounded ends, 8.9-11.3 x 3.4-4.7 µm, with straight germ slit spore-length; **perispore** dehiscent in 10% KOH, with inconspicuous coil-like ornamentation; **epispore** smooth; **colonies** on PDA covering 9-cm Petri dish in two weeks at room temperature, 25°C, at first cream-coloured white then brown, felty, azonate, with diffuse margins; **anamorph** not formed.

> SPECIMENS EXAMINED: **THAILAND**, Kanchanaburi Province, Chong Kho Neab Forest, on bamboo, 14 December 2003, Suwannasai, N. (Holotype *SUT282*); Trad Province, Ta Gum Forest, on bamboo, 19 September 2003, Phosri, C. *SUT250*.

Comments: *Hypoxylon sublenormandii* is close to *H. lenormandii* (Ju & Rogers 1996) but the stromatal surface of this new taxon is strongly reddish brown while *H. lenormandii* is grayish sepia (106), fuscous (103), or brown vinaceous (84). Asci of *H. lenormandii* are longer, 123-170 µm total length x 6-9 µm broad, than *H. sublenormandii*, which are 95-110 µm total length x 3.8-5 µm broad. The germination slit form of *H. sublenormandii* is straight spore-length whereas in *H. lenormandii* it is slightly sigmoid spore-length. Ascospores of *H. lenormandii* are slightly larger, 9.5-15 (-16) x 4-6.5 (-7) µm, than those of *H. sublenormandii*. This new taxon is known only from bamboo clumps. Ju & Rogers (1996) discussed the occurrence of *H. lenormandii* on both monocot and dicot substrates suggesting that this wide host range and the broad range of ascospore size indicate that the taxon might be subdivided into subtaxa. *Hypoxylon suranareei* would therefore be one of these and according to Ju (pers. comm.) it might in the future prove to be conspecific with *H. disjunctum* Rehm. collected originally on bamboo in Philippines.

Hypoxylon suranareei Suwannasai, Rodtong, Thienhirun & Whalley, **sp. nov.**

FIGURES **2E-H**

> *Stromata hemisphaerica vel effuso-pulvinata, tumulis peritheciorum conspicuis, externe ferruginea; sub superficie et inter perithecia granulis aurantiacis conspersa, granulis aurantiacis vel in KOH dissolutis; Perithecia globosa 0.2-0.4 mm diam. Ostiola plana vel umbilicata. Asci 90-120 µm longitudine tota x 3.8-5 µm crassa, partibus sporiferis 70-85 um, longitudine stipitibus 30-50 µm. longitudine annulo apicali in liquore iodato Melzeri cyanescente, discoideo. Ascosporae brunneolae vel brunneae, unicellulares, ellipsoideo-inequilaterales cum apicibus angustatis, (10-) 12.5-14 x 5-6.3 µm, rima germinativa recta longa praeditae; perisporium in KOH dehiscens; episprium leve.*

KEY CHARACTERS: **Stromata** hemispherical to effused-pulvinate, often appearing almost rosellinioid but joined by thin stromal tissue, conspicuous perithecial mounds, surface fulvous (43), or rust (39), orange granules immediately beneath surface and between perithecia, with KOH-extractable pigments orange (7) or scarlet (5); **perithecia** obovoid, 0.2-0.4 mm diam; **ostioles** same or lower than the stromatal surface, with white substance; **asci** 90-120 µm total length x 3.8-5 µm broad, the spore bearing parts

70-85 μm long with stipes 30-50 μm; **ascospores** brown to dark brown, unicellular, ellipsoid-inequilateral, with narrowly rounded ends, (10-)12.5-14 x 5-6.3 μm, with straight germ slit spore-length; **perispore** dehiscent in 10% KOH, with inconspicuous coil-like ornamentation; **epispore** smooth; **colonies** on PDA covering 9-cm Petri dish in two weeks at room temperature, 25°C, at first cream-coloured white then pale grayish, alternate rings, velvety, with diffuse margins; **anamorph** not formed.

> **SPECIMENS EXAMINED:** THAILAND, Nakhon Ratchasima Province, Suranaree University of Technology, on White Popinac (*Leucaena leucocephalade* Wit.), 17 November 2003, Suwannasai, N. (Holotype *SUT182*) *SUT183*, *SUT184*.

Comments: *Hypoxylon suranareei* is close to *H. anomalum* J.D. Rogers et al. (Ju et al. 2005) but their KOH-extractable pigments are different. *Hypoxylon suranareei* has orange (7) or scarlet (5) pigments but in *H. anomalum* they are luteous (12). The colour of granules beneath the surface and between the perithecia in *H. suranareei* is orange but in *H. anomalum* the colour is reddish brown. Asci of *H. anomalum* are considerably longer and broader, 140-160 μm total length x 10-11.5 μm broad, whereas in *H. suranareei* they are 90-120 μm total length x 3.8-5 μm broad. Notably, the perispore ornamentation of *H. suranareei* is inconspicuously coil-like whereas *H. anomalum* is smooth.

DNA amplification and sequence analysis

Approximately 600 to 900 base pair (bp) of the amplified ITS1-5.8S-ITS2 fragments were achieved. Details of ITS sequences of each isolate are reported in Table 3. The greatest variation in length and sequence was observed in the ITS1 region which ranged from 156 bp (*H. fendleri* SUT280) to 506 bp (*H. atroroseum* SUT009). The ITS2 region ranged from 160 bp (*H. lendormandii*) to 169 bp (*H. purpureonitens*). The length of 5.8S region was constant at 155 bp in all *Hypoxylon* isolates and highly conserved. The extremely long ITS1 sequences (477 and 506 bp) were found in both isolates of *H. stygium* (SUT058 and SUT243) and in *H. atroroseum* (SUT009 and SUT010), and this is in agreement with previous findings for *H. stygium* (AJ390409) and *H. atroroseum* (AJ390397) respectively (Sánchez-Ballesteros et al. 2000). *Hypoxylon stygium* and *H. atroroseum* appeared to be closely related as shown by their 93% identity. The ITS1-5.8S-ITS2 sequence alignment of both species revealed the insertion and/or deletion sequences of 28 bp (5′ ATCTGCTCGAATAAAATTGCTTCAATAT 3′) within ITS1 region. This sequence fragment might be useful for the designer of a probes or markers for species specific detection.

Phylogenetic relationships

Phylogenetic relationships among *Hypoxylon* specimens are presented from the neighbour-joining analysis of the aligned ITS1-5.8S-ITS2 sequences except for sequences of *H. stygium* (SUT058 and SUT243) and *H. atroroseum* (SUT009 and SUT010) because their extremely long ITS1 sequences could not be unambiguously aligned correctly. Support for specific branches of the trees was assessed by the percentage of the neighbour-joining trees of 1000 bootstrapped data sets forming the branch. In Fig. 3, the tree assigned the taxa to three main clades. Clade I consisted of two species,

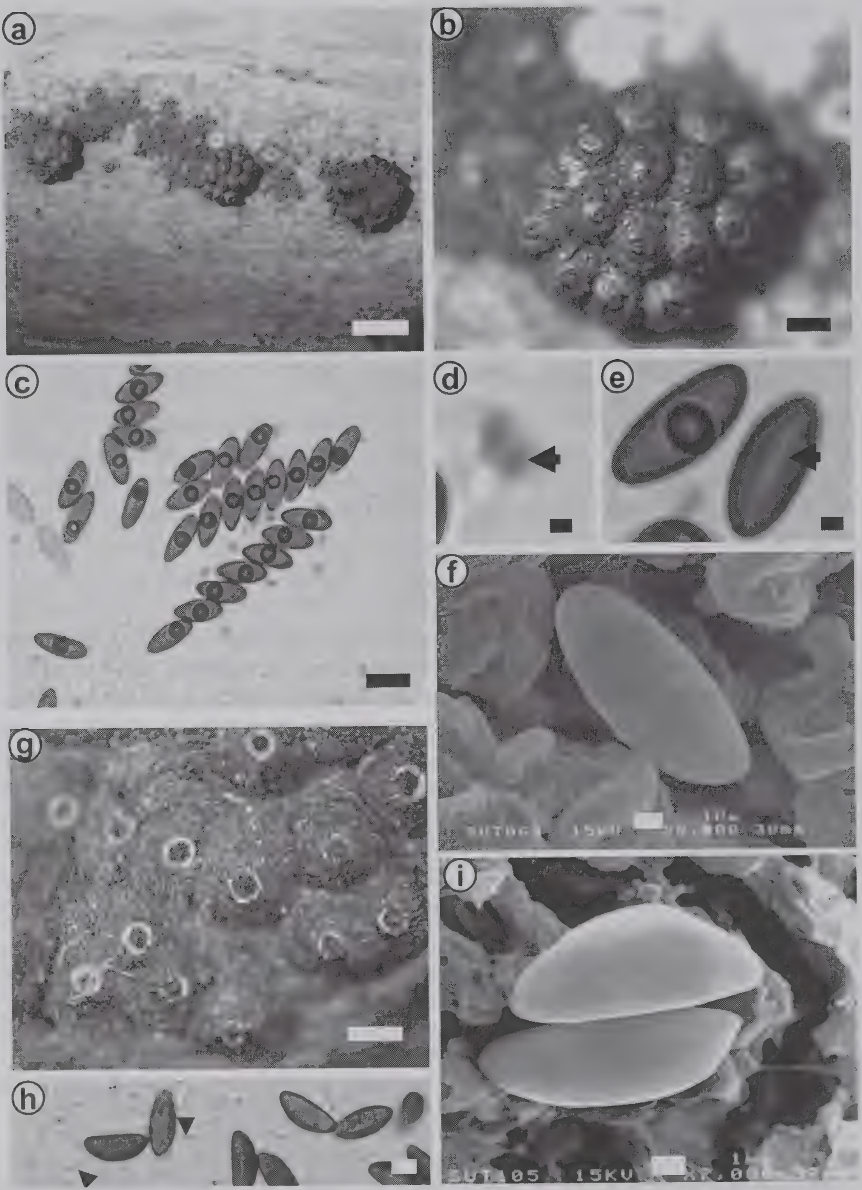


Fig. 1 *Hypoxylon kanchanapisekii* SUT069; a and b, stromatal form on bamboo (bars=1 mm and 0.2 mm respectively); c, ascospores (bar=10 μm); d, apical apparatus with discoid form (arrow) (bar=1 μm); e, straight 2/3 length of germ slit (arrow) (bar=1 μm); f, SEM micrograph of ascospore (bar=1 μm). *Hypoxylon* cf. *archeri* (SUT105); g, stromata with white fringe surrounding ostiolar disks (bar= 0.2 mm); h, ascospores with thickening of perispores (arrow) (bar=2 μm); and I, SEM micrograph of ascospores (bar=1 μm).

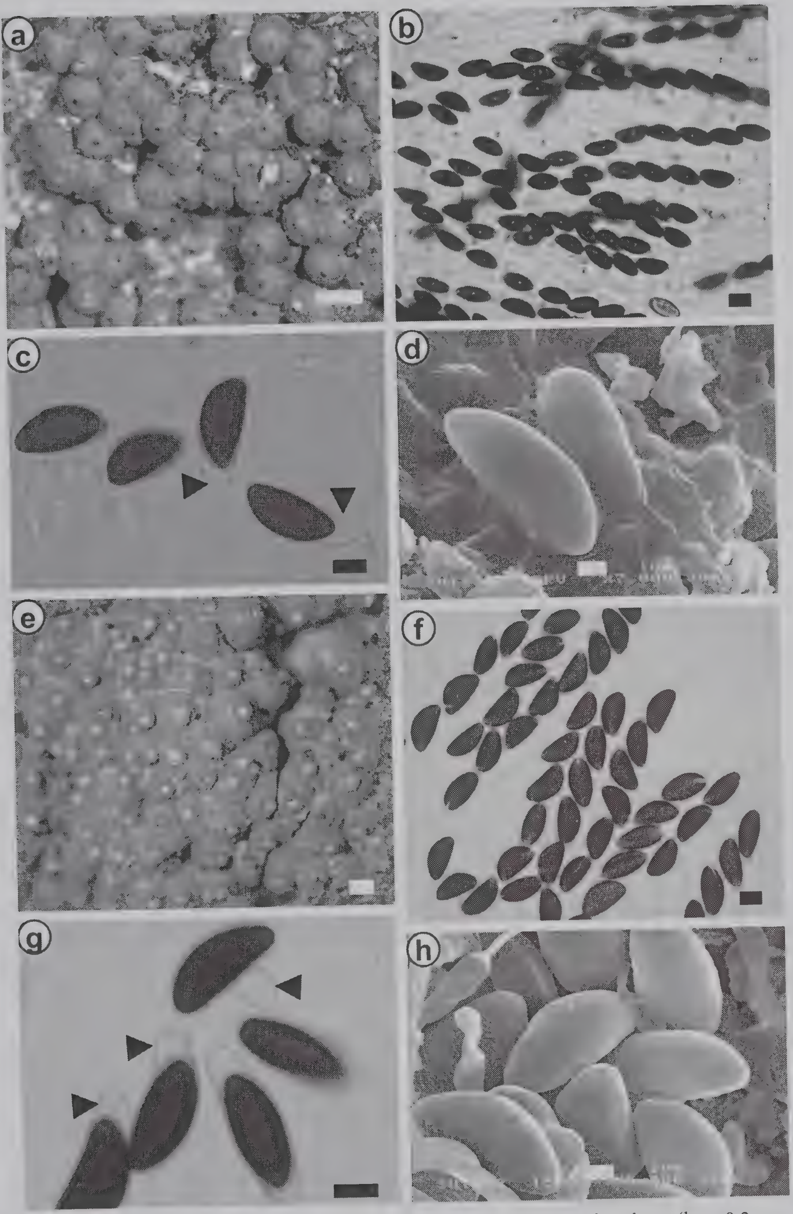


Fig. 2 *Hypoxylon sublenormandii* SUT250; a, stromatal form on bamboo (bar=0.2 mm); b, ascospores (bar=5 μm); c, ascospores with perispores dehiscing in 10% KOH (arrow) (bar=4 μm); d, SEM micrograph of ascospores (bar=1 μm). *H. suranareei* SUT182; e, stromatal form (bar=0.2 mm); f, ascospores (bar=4 μm); g, ascospores with perispores dehiscing in 10% KOH (arrow) (bar=5 μm); and h, SEM micrograph of ascospores (bar=1 μm).

Table 1. *Hypoxylon* species investigated in the present study.

No	Species	Host	Location/Source
1	*Hypoxylon anthochroum* (SUT233)	Angiosperm wood	SUT's Herbarium (specimen collected from Trad Province, Thailand)
2	*H. anthochroum* (SUT240)	Angiosperm wood	SUT's Herbarium (specimen collected from Trad Province, Thailand)
3	*H.* cf. *archeri* (SUT103)	Angiosperm wood	SUT's Herbarium (specimen collected from Songkhla Province, Thailand)
4	*H.* cf. *archeri* (SUT105)	Angiosperm wood	SUT's Herbarium (specimen collected from Songkhla Province, Thailand)
5	*H.* cf. *archeri* (ST2333)	Angiosperm wood	Royal Forest Department's Herbarium (Thailand)
6	*H.* cf. *archeri* (ST2527)	Angiosperm wood	Royal Forest Department's Herbarium (Thailand)
7	*H. atroroseum* (SUT009)	Angiosperm wood	SUT's Herbarium (specimen collected from Nakhon Ratchasima Province, Thailand)
8	*H. atroroseum* (SUT010)	Angiosperm wood	SUT's Herbarium (specimen collected from Nakhon Ratchasima Province, Thailand)
9	*H. bovei* var. *microspora* (Taiwan)	No detail	Yu-Ming Ju's Herbarium (Taiwan)
10	*H. bovei* var. *microspora* (ST2406)	Angiosperm wood	Royal Forest Department's Herbarium (Thailand)
11	*H. bovei* var. *microspora* (ST2575)	Angiosperm wood	Royal Forest Department's Herbarium (Thailand)
12	*H. fendleri* (SUT061)	Angiosperm wood	SUT's Herbarium (specimen collected from Ratchaburi Province, Thailand)
13	*H. fendleri* (SUT159)	Angiosperm wood	SUT's Herbarium (specimen collected from Yasothon Province, Thailand)
14	*H. fendleri* (SUT162)	Angiosperm wood	SUT's Herbarium (specimen collected from Yasothon Province, Thailand)
15	*H. fendleri* (SUT165)	Angiosperm wood	SUT's Herbarium (specimen collected from Yasothon Province, Thailand)
16	*H. fendleri* (SUT280)	Angiosperm wood	SUT's Herbarium (specimen collected from Kanchanaburi Province, Thailand)
17	*H. haematostroma* (SUT164)	Angiosperm wood	SUT's Herbarium (specimen collected from Yasothon Province, Thailand)
18	*H. haematostroma* (SUT292)	Angiosperm wood	SUT's Herbarium (specimen collected from Kanchanaburi Province, Thailand)
19	*H. haematostroma* (SUT293)	Angiosperm wood	SUT's Herbarium (specimen collected from Kanchanaburi Province, Thailand)
20	*H. kanchanapisekii* (SUT066)	Bamboo clump	SUT's Herbarium (specimen collected from Ratchaburi Province, Thailand)
21	*H. kanchanapisekii* (SUT068)	Bamboo clump	SUT's Herbarium (specimen collected from Ratchaburi Province, Thailand)
22	*H. kanchanapisekii* (SUT069)	Bamboo clump	SUT's Herbarium (specimen collected from Ratchaburi Province, Thailand)
23	*H. lenormandii* (SUT016)	Angiosperm wood	SUT's Herbarium (specimen collected from Burirum Province, Thailand)
24	*H. lenormandii* (SUT180)	Angiosperm wood	SUT's Herbarium (specimen collected from Nakhon Ratchasima Province, Thailand)
25	*H. lenormandii (ST2324)*	Angiosperm wood	Royal Forest Department's Herbarium (Thailand)
26	*H. monticulosum (SUT042)*	Angiosperm wood	SUT's Herbarium (specimen collected from Ratchaburi Province, Thailand)

Note: SUT Suranaree = University of Technology.

Table 1 concluded

No	Species	Host	Location/Source
27	*H. monticulosum* (SUT080)	Angiosperm wood	SUT's Herbarium (specimen collected from Nakhon Ratchasima Province, Thailand)
28	*H. monticulosum* (SUT116)	Angiosperm wood	SUT's Herbarium (specimen collected from Songkhla Province, Thailand)
29	*H. nitens* (Taiwan)	No detail	Yu-Ming Ju's Herbarium (Taiwan)
30	*H. nitens* (ST2313)	Angiosperm wood	Royal Forest Department's Herbarium (Thailand)
31	*H. nitens* (ST2473)	Angiosperm wood	Royal Forest Department's Herbarium (Thailand)
32	*H. purpureonitens* (SUT004)	Angiosperm wood	SUT's Herbarium (specimen collected from Nakhon Ratchasima Province, Thailand)
33	*H. purpureonitens* (SUT167)	Angiosperm wood	SUT's Herbarium (specimen collected from Yasothon Province, Thailand)
34	*H. purpureonitens* (SUT262)	Angiosperm wood	SUT's Herbarium (specimen collected from Trad Province, Thailand)
35	*H. purpureonitens* (ST2448)	Angiosperm wood	Royal Forest Department's Herbarium (Thailand)
36	*H. purpureonitens* (ST2485)	Angiosperm wood	Royal Forest Department's Herbarium (Thailand)
37	*H. rubiginosum* (SUT148)	Angiosperm wood	SUT's Herbarium (specimen collected from Trad Province, Thailand)
38	*H. rubiginosum* (SUT187)	Angiosperm wood	SUT's Herbarium (specimen collected from Trad Province, Thailand)
39	*H. stygium* (SUT058)	Angiosperm wood	SUT's Herbarium (specimen collected from Ratchaburi Province, Thailand)
40	*H. stygium* (SUT243)	Angiosperm wood	SUT's Herbarium (specimen collected from Trad Province, Thailand)
41	*H. sublenormandii* (SUT250)	Bamboo clump	SUT's Herbarium (specimen collected from Trad Province, Thailand)
42	*H. sublenormandii* (SUT282)	Bamboo clump	SUT's Herbarium (specimen collected from Kanchanaburi Province, Thailand)
43	*H. suranareei* (SUT182)	White Popinac (*Leucaena leucocephalade* Wit.)	SUT's Herbarium (specimen collected from Nakhon Ratchasima Province, Thailand)
44	*H. suranareei* (SUT183)	White Popinac (*Leucaena leucocephalade* Wit.)	SUT's Herbarium (specimen collected from Nakhon Ratchasima Province, Thailand)

Note: SUT Suranaree = University of Technology.

Table 2. Morphological characteristics of the *Hypoxylon* species investigated.

Species	Stromatal surface color	KOH-extractable pigment color	Ostiolar disc	Ascospore	Germ Slit	Perispore	Anamorph
Hypoxylon sect. *Annulata*							
H. cf. *archeri* Berk.	Blackish brown with white fringe	Hazel	*Truncatum*-type, 0.1-0.2 mm diameter	Ellipsoid-inequilateral, with narrowly rounded ends, 8.8-10(-11.5) x 3.8-5 µm	Straight full length	Smooth	*Nodulisporium*-like
H. atroroseum J.D. Rogers	Vinaceous gray	Greenish olivaceous	*Truncatum*-type, 0.1-0.2 mm diameter	Ellipsoid-equilateral, with narrowly rounded ends, 6.3-8.8 x 2.5-3.8 µm	Straight full length	Smooth	*Nodulisporium*-like
H. bovei var. *microspora* J.H. Miller	Black	Greenish olivaceous	*Bovei*-type, 0.3-0.7 mm diameter	Ellipsoid-inequilateral, with narrowly rounded ends, 7.5-10 x 3.8-5 µm	Straight full length	Smooth	*Nodulisporium*-like
H. nitens (Ces.) Y.-M. Ju & J.D. Rogers	Black	Greenish olivaceous	*Bovei*-type, 0.3-0.4 mm diameter	Ellipsoid-inequilateral, with narrowly rounded ends, 7.5-10 x 3.8-5 µm	Straight full length	Smooth	*Nodulisporium*-like
H. purpureonitens Y.-M. Ju & J.D. Rogers	Blackish with reddish brown tone, some shiny black	Vinaceous purple	*Bovei*-type, 0.3-0.4 mm diameter	Ellipsoid-inequilateral, with narrowly rounded ends, 7.5-10 x 3.8-5 µm	Straight full length	Smooth	*Nodulisporium*-like
H. stygium (Lév.) Sacc.	Blackish with reddish brown tone	Greenish olivaceous	*Truncatum*-type, 0.1-0.2 mm diameter	Ellipsoid-equilateral, with narrowly rounded ends, 3.8-6.3 x 2.5-3.8 µm	Straight full length	Smooth	*Periconiella*-like

Table 2, continued

Species	Stromatal surface color	KOH-extractable pigment color	Ostiolar disc	Ascospore	Germ Slit	Perispore	Anamorph
Hypoxylon sect. *Hypoxylon*							
H. anthochroum Berk. & Broome	Chestnut or brown vinaceous	Olivaceous	Lower than the stromatal surface	Ellipsoid-inequilateral, with narrowly rounded ends, 10.8-13(-14) x 4-6 µm	Straight full length	Dehiscent, with inconspicuous coil-like ornamentation	*Nodulisporium*-like
H. fendleri Berk. ex Cooke	Brown vinaceous or dark brick	Orange	Lower than the stromatal surface	Ellipsoid-inequilateral, with narrowly rounded ends, 8.5-11.5(-12.5) x 3.5-5 µm	Slightly sigmoid full length	Dehiscent, with inconspicuous coil-like ornamentation	*Nodulisporium*-like
H. haematostroma Mont.	Orange or rust	Orange	Lower than the stromatal surface	Ellipsoid-inequilateral, with narrowly rounded ends, 13-17.9 x 6.3-8.6 µm	Slightly sigmoid full length	Dehiscent, smooth	*Periconiella*-like
H. kanchanapisekii sp. nov.	Dull reddish brown	brown vinaceous , umber	slightly higher or the same as the stromatal surface	Ellipsoid-equilateral, with narrowly rounded ends, 10-11.5(-12.5) x (1-)3.5-5 µm	Straight full length	Indehiscent, smooth	Unknown
H. lenormandii Berk. & M.A. Curtis	Grayish sepia	Red	Slightly higher than the stromatal surface	Ellipsoid-inequilateral to equilateral, with narrowly rounded ends, 10-12.5 x 3.8-5 µm	Slightly sigmoid full length	Dehiscent, with inconspicuous coil-like ornamentation	*Nodulisporium*-like

Table 2, concluded

Species	Stromatal surface color	KOH-extractable pigment color	Ostiolar disc	Ascospore	Germ Slit	Perispore	Anamorph
Hypoxylon sect. *Hypoxylon*							
H. monticulosum Mont.	Rust, brown vinaceous then blackish when mature	Colorless or purple	Higher than the stromatal surface and minutely papillate	Ellipsoid-inequilateral, with narrowly rounded ends, (6.3-)7.5-8.8 (-11.3) x 3.8-5µm	Slightly sigmoid full length	Dehiscent, with smooth to inconspicuous coil-like ornamentation	Unknown
H. rubiginosum (Pers.) Fr.	Brown vinaceous	Rust	Lower than the stromatal surface	Ellipsoid-inequilateral, with narrowly rounded ends, (7.5-)8.8-10 x 3.8-5 µm	Straight full length	Dehiscent, with smooth to inconspicuous coil-like ornamentation	*Nodulisporium*-like
H. sublenormandii sp. nov.	Reddish brown	Brown vinaceous or umber	Slightly higher than the stromatal surface	Ellipsoid-inequilateral to equilateral, with narrowly rounded ends, 9-12 x 3.8-5 µm	Straight full length	Dehiscent, with inconspicuous coil-like ornamentation	Unknown
H. suranareei sp. nov.	Fulvous or rust	Orange or scarlet	Same or lower than the stromatal surface, with white substance	Ellipsoid-inequilateral, with narrowly rounded ends, (10-)12.5-14x 5-6.3 µm	Straight full length	Dehiscent, with inconspicuous coil-like ornamentation	Unknown

H. monticulosum (SUT042, SUT080, and SUT116) and *H. suranareei* (SUT182 and SUT183). Two representatives of *H. monticulosum* isolates, SUT042 and SUT080, were without apparent KOH-extractable pigments as detailed by Ju & Rogers (1996) whereas *H. monticulosum* SUT116 had a purple coloured extract. This does however agree with Ju & Rogers (1996) who stated that "it is noteworthy that the purplish stromatal pigments dark livid to dark vinaceous of *H. monticulosum* and *H. submonticulosum* are easily detected in the young, rusty brown stromata but are hardly so in the mature, blackened stromata". The sequence alignment indicated 99% similarity and it is concluded that they represent the same taxon regardless of extractable pigment in KOH. *H. suranareei* (SUT182 and SUT183) isolates were separated from *H. monticulosum* with high bootstrap support, which confirmed this new taxon.

Clade II contained three *Annulata* species, *H. nitens* (Taiwan, ST2475 and ST2313), *H. bovei* var. *microspora* (Taiwan, ST2406 and ST2579), and *H. purpureonitens* (SUT004, SUT167, SUT262, ST2448, and ST2485). Additionally six species consisting of *H. lenormandii* (SUT016, SUT180, and ST2324), *H. sublenormandii* (SUT250 and SUT282), *H. haematostroma* (SUT164, SUT292, and SUT293), *H. anthochroum* (SUT233 and SUT240), *H. kanchanapisekii* (SUT066, SUT068, and SUT069), and *H. rubiginosum* (SUT215 and SUT221), were also grouped together. The three *Annulata* species are complex species and are very similar in morphological characteristics. The two isolates of *H. nitens* from Thailand exhibited a 95% similarity to *H. nitens* from Taiwan but they were closely linked, and they are therefore considered to be the same. *Hypoxylon purpureonitens* showed 28% divergence to *H. nitens*. This is in agreement with Ju and Rogers (1996) who reported that *H. purpureonitens* is very close to *H. nitens* in its teleomorphic morphology except KOH-extractable pigments. *Hypoxylon purpureonitens* is purple or livid whereas *H. nitens* is greenish olivaceous in 10% KOH.

Three isolates of *H. lenormandii* (ST2324, SUT016, and SUT180) closely matched the description by Ju & Rogers (1996) and all collections grew on dicotyledonous wood from different forest areas as detailed in Table 1. They were clearly separated from *H. sublenormandii* (SUT250 and SUT282), with both collections occurring on bamboo, with high bootstrap support (Fig. 3). They also differed in morphological characters such as spore size (9-12 x 3.8-5 µm), a more reddish brown stromatal surface color, and a straight germ slit and on the basis of this and the sequence data a new species is proposed. Three isolates of *H. haematostroma* (SUT164, SUT292, and SUT293) could also be separated from the other taxa in the same clade because of their distinctive teleomorphic characteristics having red or orange red stromatal granules. *Hypoxylon anthochroum* (SUT233 and SUT240), *H. kanchanapisekii* (SUT066, SUT068, and SUT069), and *H. rubiginosum* (SUT215 and SUT221) were separated from each other with high bootstrap support. Although *H. anthochroum* was considered to be a synonym of *H. rubiginosum* by Miller (1961), they differ in KOH-extractable pigment color. This phylogenetic tree also confirmed the separate identity of a further new species, *H. kanchanapisekii*, which was separated from other species examined.

Clade III consisted of three species, *H. fendleri* (AJ390400), *H.* cf. *fendleri* (SUT061, SUT159, SUT162, SUT165, and SUT280) and *H.* cf. *archeri* (SUT103, SUT105, ST2333, and ST2527). Initially, five *H.* cf. *fendleri* (SUT061, SUT159, SUT162, SUT165, and SUT280) collections had been identified as *H. fendleri* since their morphological

Table 3. Sizes of ITS1 and ITS2 regions and 5.8S ribosomal nucleotide sequences.

No.	Species	ITS1 (bp)	5.8S (bp)	ITS2 (bp)	Total (bp)	GenBank Accession number
1	*Hypoxylon anthochroum* (SUT233)	180	155	162	497	QD201125
2	*H. anthochroum* (SUT240)	180	155	162	497	QD201126
3	*H.* cf. *archeri* (SUT103)	209	155	161	525	QD201121
4	*H.* cf. *archeri* (SUT105)	209	155	161	525	QD201122
5	*H.* cf. *archeri* (ST2333)	209	155	161	525	QD201123
6	*H.* cf. *archeri* (ST2527)	224	155	160	539	QD201124
7	*H. atroroseum* (SUT009)	506	155	164	825	DQ223733
8	*H. atroroseum* (SUT010)	506	155	164	825	DQ223734
9	*H. bovei* var. *microspora* (Taiwan)	226	155	167	548	QD201127
10	*H. bovei* var. *microspora* (ST2406)	225	155	167	547	QD201128
11	*H. bovei* var. *microspora* (ST2579)	226	155	167	548	QD201129
12	*H. fendleri* (SUT061)	180	155	165	500	QD201130
13	*H. fendleri* (SUT159)	183	155	164	502	QD201132
14	*H. fendleri* (SUT162)	183	155	165	503	DQ223735
15	*H. fendleri* (SUT165)	182	155	163	500	DQ223736
16	*H. fendleri* (SUT280)	156	155	163	474	DQ223737
17	*H. haematostroma* (SUT164)	176	155	161	492	DQ223738
18	*H. haematostroma* (SUT292)	176	155	161	492	DQ223739
19	*H. haematostroma* (SUT293)	176	155	161	492	DQ223740
20	*H. kanchanapisekii* (SUT066)	209	155	162	526	DQ223741
21	*H. kanchanapisekii* (SUT068)	209	155	162	526	DQ223742
22	*H. kanchanapisekii* (SUT069)	209	155	162	526	DQ223743
23	*H. lenormandii* (SUT016)	188	155	160	503	DQ223744
24	*H. lenormandii* (SUT180)	188	155	160	503	DQ223745
25	*H. lenormandii* (ST2324)	188	155	160	503	DQ223746
26	*H. monticulosum* (SUT042)	171	155	165	491	DQ223747
27	*H. monticulosum* (SUT080)	171	155	165	491	DQ223748
28	*H. monticulosum* (SUT116)	171	155	165	491	DQ223749
29	*H. nitens* (Taiwan)	158	155	166	479	DQ223750
30	*H. nitens* (ST2313)	158	155	166	479	DQ223751
31	*H. nitens* (ST2473)	158	155	166	479	DQ223752
32	*H. purpureonitens* (SUT004)	225	155	169	549	DQ223753
33	*H. purpureonitens* (SUT167)	225	155	169	549	DQ223754
34	*H. purpureonitens* (SUT262)	225	155	169	549	DQ223755
35	*H. purpureonitens* (ST2448)	225	155	169	549	DQ223756
36	*H. purpureonitens* (ST2485)	225	155	169	549	DQ223757
37	*H. rubiginosum* (SUT215)	178	155	164	497	DQ223758
38	*H. rubiginosum* (SUT221)	178	155	164	497	DQ223759
39	*H. stygium* (SUT058)	477	155	164	796	DQ223760
40	*H. stygium* (SUT243)	477	155	164	796	DQ223761
41	*H. sublenormandii* (SUT250)	198	155	161	514	DQ223762
42	*H. sublenormandii* (SUT282)	198	155	161	514	DQ223763
43	*H. suranareei* (SUT182)	199	155	162	516	DQ223764
44	*H. suranareei* (SUT183)	199	155	162	516	DQ223765

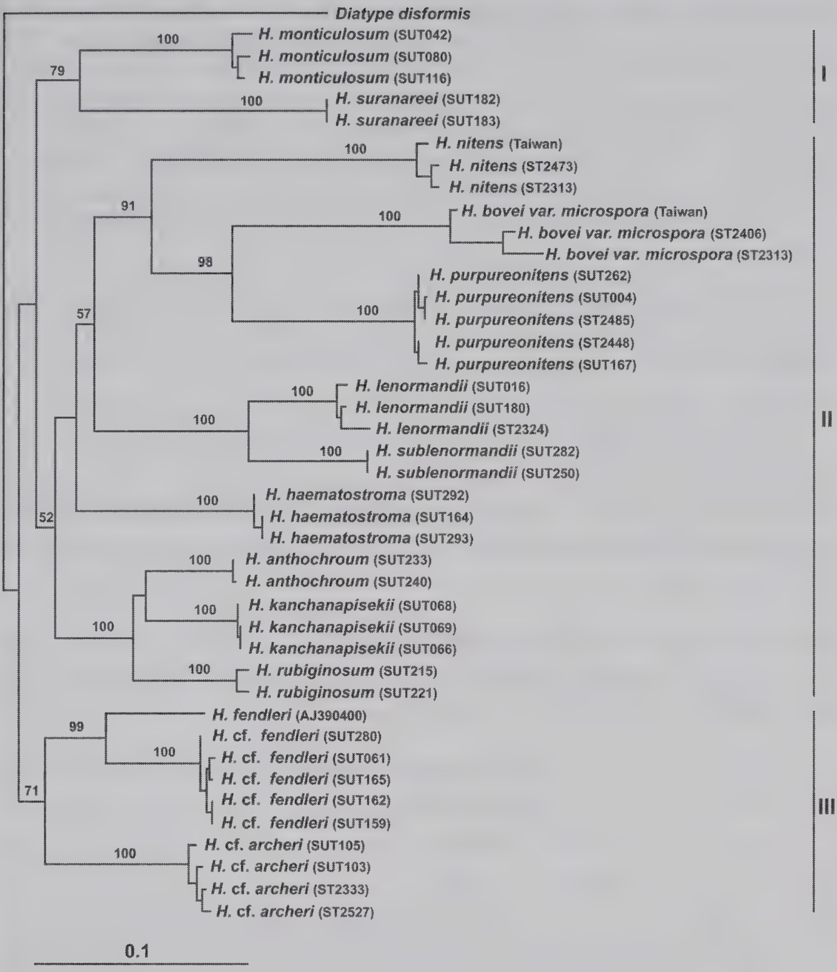

Diatype disformis
H. monticulosum (SUT042)
H. monticulosum (SUT080)
H. monticulosum (SUT116)
H. suranareei (SUT182)
H. suranareei (SUT183)
H. nitens (Taiwan)
H. nitens (ST2473)
H. nitens (ST2313)
H. bovei var. microspora (Taiwan)
H. bovei var. microspora (ST2406)
H. bovei var. microspora (ST2313)
H. purpureonitens (SUT262)
H. purpureonitens (SUT004)
H. purpureonitens (ST2485)
H. purpureonitens (ST2448)
H. purpureonitens (SUT167)
H. lenormandii (SUT016)
H. lenormandii (SUT180)
H. lenormandii (ST2324)
H. sublenormandii (SUT282)
H. sublenormandii (SUT250)
H. haematostroma (SUT292)
H. haematostroma (SUT164)
H. haematostroma (SUT293)
H. anthochroum (SUT233)
H. anthochroum (SUT240)
H. kanchanapisekii (SUT068)
H. kanchanapisekii (SUT069)
H. kanchanapisekii (SUT066)
H. rubiginosum (SUT215)
H. rubiginosum (SUT221)
H. fendleri (AJ390400)
H. cf. fendleri (SUT280)
H. cf. fendleri (SUT061)
H. cf. fendleri (SUT165)
H. cf. fendleri (SUT162)
H. cf. fendleri (SUT159)
H. cf. archeri (SUT105)
H. cf. archeri (SUT103)
H. cf. archeri (ST2333)
H. cf. archeri (ST2527)
100
79
100
100
91
100
98
100
57
100
100
100
100
52
100
100
100
100
99
100
71
100
I
II
III
0.1

Fig. 3 Phylogenetic tree construction based on ITS1-5.8S-ITS2 sequences of *Hypoxylon* species. Branch lengths are scaled in terms of expected numbers of nucleotide substitution per site. Numbers on branches are bootstrap values from 1000 replications.

characteristics matched *H. fendleri* (Ju and Rogers 1996). However, Ju and Rogers (1996) pointed out that *H. fendleri* and *H. retpela* Van der Gucht & Van der Veken are very similar stating "These two fungi differ mainly in the conspicuousness of their perispore ornamentation". The ornamentation in *H. retpela* is described as very conspicuously coil-like. However all the Thai collections had similar coiling which was not noticeably conspicuous. Thus the description for *H. fendleri* (Ju & Rogers 1996) is the nearest match. The phylogenetic result showed that all the Thai isolates (SUT061, SUT159, SUT162, SUT165, and SUT280) grouped together and were placed as a sister branch of *H. fendleri* (AJ390400) based on the GenBank database sequence with high bootstrap support. The percentage similarity of *H. fendleri* (AJ390400) to SUT061, SUT159, SUT162, SUT165, and SUT280 isolates was 85%, 85%, 85%, 85%, and 80% respectively. They are therefore quite different and as a result the Thai collections were recorded as *H.* cf. *fendleri*. This might be the result of a wide range of *H. fendleri* descriptions (morphological) or genetic variation within this taxon found in Thailand. More collections of specimens around the world are required for a better understanding of species delimitation and genetic variation within this taxon.

Four isolates of *H.* cf. *archeri* were grouped together. All of them had a small ostiolar disc around 0.1-0.2 mm in diameter, and belonged to the section *Annulata*. They appeared as a paraphyletic group with other isolates of the section *Annulata* also placed in Clade II. Its morphological features were similar to those of *H. archeri* Berk. and *H. michelianum* Ces. & De Not. Ascospore dimensions, stromatal form, and coloration are indicative of *H. archeri*, but the distinctive white fringe surrounding the ostioles is reminiscent of *H. michelianum*. However, *H.* cf. *archeri* examined, in the current study, grouped in Clade III and were thus separated from the other species of the *Annulata*, which grouped in Clade II. Unfortunately, no voucher material was available for DNA extraction or for morphological comparison, and no sequences were available in GenBank.

Key to taxa of section *Hypoxylon* in this study

1a. Ascospores with perispore not dehiscent in 10% KOH ..... ***H. kanchanapisekii***
1b. Ascospores with perispore dehiscent in 10% KOH 2

2a. (1b) Stromatal surface without vinaceous shades 3
2b. Stromatal surface with vinaceous shades 6

3a. (2a) Ascospores less than 12 µm long 4
3b. Ascospores greater than 12 µm long 5

4a. (3a) Stromatal glomerate to effused-pulvinate, rosellinioid; grayish sepia (106), or brown vinaceous (84), with KOH-extractable pigments sienna (8), cinnamon (62), fulvous (43); ascospores brown to dark brown, unicellular, ellipsoid-inequilateral, with slightly sigmoid germ slit spore-length, 10-12.5 x 3.8-5 µm .... ***H. lenormandii***
4b. Stromatal glomerate to effused-pulvinate, rosellinioid; reddish brown, with KOH-extractable pigments brown vinaceous (84), or umber (9); ascospore brown to dark brown, unicellular, ellipsoid-inequilateral, with straight germ slit spore-length, 9-12 x 3.8-5 µm .... ***H. sublenormandii***

5a. (3b) Stromatal hemispherical to effused-pulvinate, plane or with inconspicuous to conspicuous perithecial mounds; surface orange (7), or rust (39); orange red granules immediately beneath surface and between perithecia, with KOH-extractable pigments orange (7) or scaret (5); ascospores brown to dark brown, unicellular, ellipsoid-inequilateral, with slightly sigmoid germ slit spore-length, 13-17.9 x 6.3-8.6 µm; perispore dehiscent in 10% KOH, smooth .... ***H. haematostroma***

5b. Stromatal hemispherical to effused-pulvinate, with conspicuous perithecial mounds; surface fulvous (43), or rust (39); rust granules immediately beneath surface and between perithecia, with KOH-extractable pigments orange (7) or scaret (5); ascospores brown to dark brown, unicellular, ellipsoid-inequilateral, with straight germ slit spore-length, (10-) 12.5-14 x 5-6.3 µm; perispore dehiscent in 10% KOH, inconspicuous coil-like ornamentation .... ***H. suranareei***

6a. (2b) KOH-extractable pigments olivaceous (48), or gray olivaceous (107), or greenish olivaceous (90) .... ***H. anthochroum***

6b. KOH-extractable pigments other than greenish or olivaceous, or without apparent pigments..7

7a. (6b) KOH-extractable pigments purplish or without apparent pigments .... ***H. monticulosum***

7b. KOH-extractable pigments orange (7), or rust (39) 8

8a. (7b) KOH-extractable pigments orange (7); ascospores brown to dark brown, unicellular, ellipsoid-inequilateral, with slightly sigmoid germ slit spore-length, 8.5-11.5 (-12.5) x 3.8-5 µm .... ***H. fendleri***

8b. KOH-extractable pigments rust (39); ascospores brown to dark brown, unicellular, ellipsoid-inequilateral, with straight germ slit spore-length, (7.5-) 8.8-10 x 3.8-5 µm ....***H. rubiginosum***

Key to taxa of section *Annulata* in this study

1a. Ostiolar disc not exceeding than 0.3 mm diameter..........................2

1b. Ostiolar disc exceeding than 0.3 mm diameter 4

2a. (1a) KOH-extractable pigments hazel (88) or honey (64) .... ***H.* cf. *archeri***

2b. KOH-extractable pigments olivaceous (48) or greenish olivaceous (90) 3

3a. (2b) Stromata blackish; conidiogenous structure *Periconiella*-like ....***H. stygium***

3b. Stromata rose-coloured with blackish; conidiogenous structure *Nodulisporium*-like .... ***H. atroroseum***

4a. (1b) Ostiolar disc variable, 0.3-0.7 mm; ascospores 7.5-10 x 3.5-5 µm .... ***H. bovei* var. *microspora***

4b. Ostiolar disc 0.2-0.5 mm; mature stromata shiny black 5

5a. (4b) KOH-extractable pigments greenish olivaceous (90); ascospores 7.5-10 x 3.8-5 µm .... ***H. nitens***

5b. KOH-extractable pigments vinaceous purple (101); ascospores 7.5-10 x 3.8-5 µm .... ***H. purpureonitens***

Conclusions

Fifteen *Hypoxylon* species identified from eighty nine collections from Thailand were recorded including three new species, *H. kanchanapisekii*, *H. sublenormandii*, and *H. suranareei*. The phylogenetic relationships between morphological and molecular data of the *Hypoxylon* species were achieved following sequence analysis of ITS1-5.8S-ITS2 rDNA regions. These molecular results showed clear separation within the *Hypoxylon* species studied and could be used to confirm the new species. In addition, molecular data could be used to resolve taxonomic problems based solely on morphology and has the potential to recognize the delimitation of other species in future study. Within the genus molecular data is valuable for resolving species complexes and for the recognition of relationships between species. The ITS sequences obtained in the current study do not support a clear separation of members of *Hypoxylon* and section *Annulata* and this is in agreement with the findings of Sánchez-Ballesteros et al. (2000). However, Hsieh et al. (2005) provided strong evidence for their separation based on different DNA sequences which is therefore in contrast with our findings and those of Sánchez-Ballesteros et al. (2000)

Acknowledgements

This study was financially supported by the Thailand Research Fund through the Royal Golden Jubilee Ph.D. Program. Instrumental supports were provided by Suranaree University of Technology, the Royal Forest Department (Thailand), and the Biotechnology and Development Office (Department of Agriculture, Thailand). The authors are grateful to Dr. Yu-Ming Ju (National University of Taiwan) for providing some of the *Hypoxylon* strains and giving valuable comments, Asst. Prof. Dr. Saowalak Pongpaijit (Prince of Songkla University, Thailand) and Dr. Cherdchai Phosri (Pibulsongkram Rajabhat University, Thailand) for collecting *Hypoxylon* specimens, Dr. Jittra Piapukiew (Chulalongkorn University, Thailand) for phylogenetic program support, and Miss Korawan Ratanachai (Suranaree University of Technology, Thailand) for helping with the scanning electron microscopy. We greatly appreciate Prof. Roy Watling (Royal Botanic Edinburgh, U.K.) and Prof. Jack D. Rogers (Washington State University, U.S.A.) for peer reviewers.

Literature Cited

Bruns TD, White TJ, Taylor JW. 1991. Fungal molecular systematics. Annual Review of Ecology and Systematics 221: 524–564.

Edwards RL, Jonglaekha N, Kshirsagar A, Maitland DJ, Mekkamol S, Nugent LK, Phosri C, Rodtong S, Ruchikachorn N, Sangvichien E, Sharples GP, Sihanonth P, Suwannasai N, Thienhirun S, Whalley AJS, Whalley MA. 2003. The *Xylariaceae* as phytopathogens. Recent Research Developments in Plant Sciences 1: 1–19.

Felsenstein J. 1995. PHYLIP: Phylogeny inference package. Version 3.6. Department of Genetics, University of Washington.

Gardes M, Bruns TD. 1993. ITS primers with enhanced specificity for basidiomycetes application to the identification of mycorrhizae and rusts. Molecular Ecology 2: 113–118.

González FSM, Rogers JD. 1993. *Biscogniauxia* and *Camillea* in Mexico. Mycotaxon 47: 229–258.

Hsieh H-M, Ju Y-M, Rogers JD. 2005. Molecular phylogeny of *Hypoxylon* and closely related genera. Mycologia 97: 844–865.

Ju Y-M, Rogers JD. 1994. *Kretzschmariella culmorum* (Cooke) Y.-M. Ju & J.D. Rogers and notes on some other monocot-inhabiting xylariaceous fungi. Mycotaxon 51: 241–255.

Ju Y-M, Rogers JD. 1996. A revision of the genus *Hypoxylon*. Mycologia Memoir 20: 1-365. APS Press, St Paul.

Ju Y-M, Rogers JD, Hsieh HM. 2004. New *Hypoxylon* species and notes on some names associated with or related to *Hypoxylon*. Mycologia 96: 154–161.

Ju Y-M, Rogers JD, Hsieh HM. 2005. New *Hypoxylon* and *Nemania* species from Costa Rica and Taiwan. Mycologia 97: 562–567.

Ju Y-M, Rogers JD, San Martin F, Grammo A. 1998. The genus *Biscogniauxia*. Mycotaxon 66: 1–98.

Kimura M. 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. Journal of Molecular Evolution 16: 111–120.

Læssøe T, Rogers JD, Whalley AJS. 1989. *Camillea*, *Jongiella* and light-spored species of *Hypoxylon*. Mycological Research 93: 121–155.

Læssøe T, Spooner BM. 1994. *Rosellinia* and *Astrocystis* (*Xylariaceae*): new species and generic concepts. Kew Bulletin 49: 1–70.

Lee SB, Taylor JW. 1990. Isolation of DNA from fungal mycelia and single spores. In: MA. Innis, DH. Gelfand, JS. Sninsky, TJ. White, eds. PCR Protocols. Academic Press, San Diego New York Boston (1990). p. 282–287.

LoBuglio KF, Taylor JW. 1995. Phylogeny and PCR identification of the human pathogenic fungus *Penicillium marneffei*. Journal of Clinical Microbiology 33: 85–89.

Martin P. 1968. Studies in the *Xylariaceae*, IV. *Hypoxylon* sections *Papillata* and *Annulata*. J S Afr Bot 34: 303–330.

Mazzaglia A, Anselmi N, Gasbarri A, Vanini A. 2001. Sequence analysis of the 5.8S rDNA and ITS regions in evaluating genetic relationships among some species of *Hypoxylon* and related genera. Mycological Research 105: 670–675.

Miller JH. 1961. A monograph of the world species of *Hypoxylon*. University of Georgia Press: Athens, U.S.A.

O'Donnell K. 1992. Ribosomal DNA internal transcribed spacers are highly divergent in the phytopathogenic ascomycetes *Fusarium sambucinum* (*Gibberella pulicaris*). Current Genetics 22: 213–220.

Petrini LE. 1992. *Rosellinia* species of the temperate zones. Sydowia 44: 169-281.

Pouzar Z. 1985. Reassessment of *Hypoxylon serpens* –complex I. Česká Mykologia 39: 129–134.

Rayner, R.W. (1970). A mycological colour chart. Commonwealth Mycological Institute, Kew and British Mycological Society. 34 p. + charts I & II.

Rodrigues KF, Petrini, O. 1997. Biodiversity of endophytic fungi in Tropical regions. In: KD. Hyde, ed. Biodiversity of Tropical Microfungi. Hong Kong University Press, Hong Kong. p. 57–69.

Rogers JD. 1979. The *Xylariaceae*: systematic, biological and evolutionary aspects. Mycologia 71: 1–42.

Rogers JD. 2000. Thoughts and musings on tropical *Xylariaceae*. Mycological Research 104: 1412–1420.

Rogers JD, Ju Y-M. 1996. *Entoleuca mammata* comb. nov. for *Hypoxylon mammatum* and the genus *Entoleuca*. Mycotaxon 59: 441–448.

Saenz GS, Taylor JW. 1999. Phylogeny of the Erysiphales (powdery mildews) inferred from internal transcribed spacer ribosomal DNA sequences. Canadian Journal of Botany 77: 150–169.

Sánchez-Ballesteros J, González V, Salazar O, Acero J, Portal MA, Julián M, Rubio V, Bills GF, Polishook JD, Platas G, Mochales S, Peláez F. 2000. Phylogenetic study of *Hypoxylon* and related genera based on ribosomal ITS sequences. Mycologia 92: 964–977.

Schulz B, Boyle C, Draeger S, Römmert AK, Krohn K. 2002. Endophytic fungi: a source of novel biologically active secondary metabolites. Mycological Research 106: 996-1004.

Skouboe P, Frisvad JC, Taylor JW, Lauritsen D, Boysen M, Rossen L. 1999. Phylogenetic analysis of nucleotide sequences from the ITS region of terverticilattate *Penicillium* species. Mycological Research 103: 873-881.

Swofford DL. 2002. PAUP*: phylogenetic analysis using parsimony (*and other methods). Version 4. Sinauer Associates, Suderland, MA.

Thienhirun S. 1997. A Preliminary Account of the *Xylariaceae* of Thailand: Ph.D Thesis, Liverpool John Moores University, UK.

Tompson JD, Higgins DG, Gibson TJ. 1994. CLUSTALW: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nuclei Acids Research 22: 4673-4680.

Van der Gucht K. 1994. The *Xylariaceae* of Papua New Guinea. Ph.D Thesis, Univercity of Gent, Belgium.

Whalley AJS, 1996. The Xylariaceous way of life. Mycological Research 100: 897–922.

Whalley AJS., Edwards, RL. 1995. Secondary metabolites and systematic arrangement within the *Xylariaceae*. Canadian Journal of Botany 73 Suppl. 1: S802-S810.

Whalley AJS, Læssøe T, Kile GA. 1990. A new species of *Biscogniauxia* with appendaged ascospores from Tasmania. Mycological Research 94: 27–239.

White TJ, Bruns TD, Lee SB, Taylor JW. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: MA. Innis, DH. Gelfand, JS. Sninsky, TJ. White, eds. PCR Protocols. Academic Press, San Diego New York Boston (1990). pp. 315–322.

MYCOTAXON

Volume 94, pp. 325–329 October–December 2005

Tricholoma equestre, the correct name for *T. flavovirens* (*Agaricales*)

H. Deng[1, 2] & Y. -J. Yao[1*]

*yaoyj@sun.im.ac.cn

[1]Systematic Mycology and Lichenology Laboratory, Institute of Microbiology
Chinese Academy of Sciences, Beijing, 100080, China

[2]Graduate School of the Chinese Academy of Sciences

Abstract—*Tricholoma equestre* is considered to be the correct name for the species often known as *T. flavovirens*. The history of the conservation of the generic name *Tricholoma* and its type, *T. flavovirens*, is reviewed and presented here. The author citation of *T. flavovirens* is also discussed and the upcoming correction in the ICBN is addressed.

Key words—nomenclature, synonym, *Tricholomataceae*

The type of *Tricholoma* (Fr.) Staude, *nom. cons.*, is *T. flavovirens* (for the author citation of this name, see the discussion below), as reported in successive editions of the International Code of Botanical Nomenclature (ICBN; see Voss et al. 1983; Greuter et al. 1988, 1994, 2000). However, much confusion has surrounded the correct name of the species to which this name has been applied because *T. equestre* and *T. flavovirens* are almost universally regarded as synonyms. Some researchers consider *T. equestre* as the preferred name with *T. flavovirens* its synonym (e.g. Bon 1987, Tkalčec & Mešič 2002, Massart 2003, Horak 2005), but many others still accept *T. flavovirens* as the correct name and reduce *T. equestre* to synonymy (e.g. Moser 1983, Singer 1986, Phillips 1988, Hansen & Knudsen 1992, Jordan 1995, Bruns et al. 1998, Noordeloos & Christensen 1999).

In their survey of *Tricholoma* species reported from China, Deng et al. (2004) noted that both *T. equestre* (e.g. Tai 1979, Tseng 1996, CAS 1996) and *T. flavovirens* (e.g. Ying & Zang 1994, Mao 2000, Wen et al. 2001, Wang et al. 2004) were cited in the Chinese literature, with the latter used more frequently, especially in recent years.

A second, more global, literature search was conducted to determine the correct name for this species. *Agaricus equestris* (≡*T. equestre*) and *A. flavovirens* (≡*T. flavovirens*) were consistently treated as synonyms by Fries (Fries 1821, 1828, 1874). The two names were also considered synonymous both by many of Fries' contemporaries (see Saccardo 1911) and more recent agaricologists (e.g. Singer 1962, 1975, 1986; Bon 1976, 1984; Hansen & Knudsen 1992, Noordeloos & Christensen 1999, Horak 2005).

---

[*] Author for correspondence

Although *A. equestris* was first treated as a synonym of *A. flavovirens* by Fries (1821), it was restored as an accepted name and the latter reduced to a synonym (Fries 1828, and 1832 in the index to *Systema Mycologicum*). According to the ICBN (Art. 15, including Note 1, in Greuter et al. 2000), both *A. equestris* and *A. flavovirens* were sanctioned by Fries (1821, 1828), but *A. equestris*, as the earlier name, has priority over *A. flavovirens*. This conclusion is also indicated in Ex. 4 of Art. 15, ICBN, although the date of Persoon's name is incorrect (see below). Therefore, if the two are regarded as synonymous, it is clear that *T. equestre* is the correct name for the species and *T. flavovirens* is a later synonym. The author citation and references of these two names are listed below:

Tricholoma equestre (L. : Fr.) P. Kumm., Führ. Pilzk.: 130. 1871.

≡*Agaricus equestris* L., Sp. pl. Ed. 1, 1173. 1753. : Fr., Elen. fung. 1: 6. 1828.
=*Agaricus flavovirens* Pers. in Hoffmann, Abbild. Schwämme 3: t. 24. 1793. : Fr., Syst. mycol. 1: 41. 1821.
≡*Tricholoma flavovirens* (Pers. : Fr.) S. Lundell in Lundell & Nannfeldt, Fung. Exs. Suec., Fasc. 23–24: no. 1102. 1942.

Of the several proposals considered by the IAPT Special Committee for Fungi (Rogers 1953a), conservation of the generic name *Tricholoma* (Fr.) P. Kumm. 1871 against *Tricholoma* Benth. 1846 (the name of a plant genus in the *Scrophulariaceae*) was finally approved (Rogers 1953b) and formally included in the list of conserved generic names in the 1956 ICBN (Lanjouw et al. 1956) as:

> '*Tricholoma* (Fries) Kummer, Führ. Pilzk. 25. 1871.
> T.: *Agaricus flavovirens* A. & S. ex Fr. Syst. Mycol. 1: 41. 1821 (cf. *A. equestris* Fr. Elench. Fung. 1: 6. 1828).'

Donk (1962, 1968) subsequently determined that the author who first validly published the conserved *Tricholoma* was not Kummer, but Staude. Therefore, the authorship for the generic name in the 1972 ICBN (Stafleu et al. 1972) was changed from '(Fries) Kummer, Führ. Pilzk. 25, 129. 1871' to '(E. M. Fries) Staude, Pilze Mitt.-Deutschl. xviii, 25. 1857.' The type of the conserved generic name *Tricholoma*, namely '*Agaricus flavovirens* A. & S. ex Fr., Syst. Mycol. 1: 41. 1821 (cf. *A. equestris* Fr., Elench. Fung. 1: 6. 1828)', was also included in the proposal for conservation (Rogers 1953b) and accepted as thus in the 1956 edition of ICBN. Later editions of the ICBN, however, replaced the type synonymy—the part in brackets: '(cf. A. equestris Fr. Elench. Fung. 1: 6. 1828)'—with '[*A. equestris* Fries]'.

In the 1983 ICBN (Voss et al. 1983), the type was listed as '*T. flavovirens* (Albertini et Schweinitz : E. M. Fries) Lundell (*Agaricus flavovirens* Albertini et Schweinitz : E. M. Fries)', and in the most recent edition (Greuter et al. 2000) as '*Agaricus flavovirens* Alb. & Schwein. : Fr. (*T. flavovirens* (Alb. & Schwein. : Fr.) S. Lundel [sic!]). The synonym '*A. equestris*' no longer appeared in the list of conserved names. Singer, who cited *T. equestre* as the type of *Tricholoma* (Singer 1962, 1975), did list *T. flavovirens* as type in accordance with the 1983 ICBN in the 4th edition of *Agaricales* in Modern Taxonomy (Singer 1986). It is noteworthy that Bon, who first cited *T. flavovirens* with *T. equestre* as its synonym (Bon 1984), later reversed himself, citing *T. flavovirens* as synonymous to *T. equestre* (Bon 1987).

Although *T. flavovirens* has been designated as the type of *Tricholoma* in the ICBN list of conserved generic names, it is necessary to important to note that *T. equestre* is the correct name for the species to which the name *T. flavovirens* applies. In fact, it is not uncommon that the generic or specific name that indicates the type of the name of a family or genus is not the correct name for that genus or species. For example, the type of the family name *Caryophyllaceae* Juss. is *Caryophyllus* Mill. 1754 (non *Caryophyllus* L. 1753), but the accepted name for the genus to which *Caryophyllus* Mill. applies is *Dianthus* L. 1753 (Ex. 4 of Art. 18.3, ICBN).

Further, the authorship of *A. flavovirens*, 'A. & S. ex Fr.', was listed in the conservation of *Tricholoma* in the 1956 ICBN (Lanjouw et al. 1956). The authorship later changed to 'Alb. & Schwein. : Fr.' (Greuter et al. 1994, 2000), although 'Pers. (1801)' has been cited in Art. 15, Ex. 4 since 1994 (Greuter et al. 1994, 2000). However, in Fries (1821: 41), *A. flavovirens* was referred to '*Abb. d. Schw. – Pers. Syn. p.* 319.' The abbreviation *Abb. d. Schw.* stands for *Abbildungen der Schwämme*, now abbreviated as *Abbild. Schwämme* (Stafleu & Cowan 1979: 239), in which *A. flavovirens* was first published by Persoon (in Hoffmann 1793). It is unclear why this was mistaken as 'Alb. & Schw.' at least since Rogers (1953b), but the error may have arisen because the two were very well known authors who included a description of *A. flavovirens* in their major mycological publication (Albertini & Schweinitz 1805: 167). Although '*Pers. Syn. p. 319.*' (Persoon 1801) was also mentioned by Fries (1821), Persoon (1801: 319) explicitly mentioned the earlier publication of the taxon by Persoon (in Hoffmann 1793).

Accordingly, the author citation for *A. flavovirens* in both Ex. 4 of Art. 15 and the list of conserved names of the ICBN should be corrected to 'Pers. (1793)' in future editions. The authors have been informed that the authorship of the species name indicating the type of *Tricholoma* will be corrected in the upcoming ICBN (McNeill et al. 2006 in prep., Demoulin pers. comm. 2006) to: *T. flavovirens* (Pers. : Fr.) S. Lundell (*Agaricus flavovirens* Pers. : Fr.).

Acknowledgements

The authors wish to thank Drs D. N. Pegler and L. L. Norvell for serving as pre-submission reviewers. Drs R. Korf, V. Demoulin, W. Gams, S. Redhead and J. McNeill are also thanked for their valuable discussions and comments on the manuscript. Special appreciation is due to Dr S. R. Pennycook for his careful nomenclature checking and also for indication of the correct authorship for *Agaricus flavovirens*. This project is supported by the 'Programme for International Science and Technology Cooperation Project of P. R. China' (2001CB711103) from Ministry of Science and Technology, the 'General Programme Funding' (30470008) and the 'National Science Fund for Distinguished Young Scholars' (30025002) from National Natural Science Foundation of China and the 'Research Project of Innovation Programme' (KSCX2-SW-101C) and the scheme of 'Introduction of Overseas Outstanding Talents' operated by Chinese Academy of Sciences.

Literature cited

Albertini JB de, Schweinitz LD de. 1805. Conspectus Fungorum in Lusatiae Superioris Agro Niskiensi Crescentium. E Methodo Persoonian. Lipsiae: Sumtibus Kummerianis.

Bon M. 1976. Tricholomes de France et d'Europe Occidentale (4ème partie). Documents Mycologiques **6** (22–23): 165–304.

Bon M. 1984. Les Tricholomes de France et d'Europe Occidentale. Paris: Editions Lechevalier S.A.R.L.

Bon M. 1987. The Mushrooms and Toadstools of Britain and Northwestern Europe. London: Hodder & Stoughton.

Bruns TD, Szaro TM, Gardes M, Cullings, KW, Pan JJ, Taylor DL, Horton TR, Kretzer A, Garbelotto M, Li Y. 1998. A sequence database for the identification of ectomycorrhizal basidiomycetes by phylogenetic analysis. Molecular Ecology 7: 257–272.

Comprehensive Scientific Expedition Team to the Qinghai-Xizang Plateau, Chinese Academy of Sciences [CAS]. 1996. Fungi in Hengduan Mountain Region. Beijing: Science Press. (in Chinese).

Deng H, Yao Y-J, Pegler DN. 2004. An annotated checklist of *Tricholoma* from China. Journal of Fungal Research **2**: 1–18.

Donk MA. 1962. The generic names proposed for *Agaricaceae*. Beih. Nova Hedwigia **5**: 1–320.

Donk MA. 1968. Report of the committee for fungi and lichens 1964–1969. Taxon **17**: 578–582.

Fries EM. 1821. Systema Mycologicum **1**. Gryphiswaldiae: Sumtibus Ernesti Mauritii.

Fries EM. 1828. Elenchus Fungorum **1**. Gryphiswaldiae: Sumtibus Ernesti Mauritii.

Fries EM. 1832. Systema Mycologicum, Index Alphabeticus. Gryphiswaldiae: Sumtibus Ernesti Mauritii.

Fries EM. 1874. Hymenomycetes Europaei. Upsaliae: Ed. Berling.

Greuter W, Burdet HM, Chaloner WG, Demoulin V, Brolle R, Hawksworth DL, Nicolson DH, Silva PC, Stafleu FA, Voss EG, McNeill J. 1988. International Code of Botanical Nomenclature. Königstein: Koltz Scientific Books.

Greuter W, Barrie FR, Burdet HM, Chaloner WG, Demoulin V, Hawksworth DL, Jørgensen PM, Nicolson DH, Silva PC, Trehane P, McNeill J. 1994. International Code of Botanical Nomenclature. Königstein: Koltz Scientific Books.

Greuter W, McNell J, Barrie FR, Burdet HM, Demoulin V, Filgueiras TS, Nicolson DH, Silva PC, Skog JE, Trehane P, Turland NJ, Hawksworh DL. 2000. International Code of Botanical Nomenclature. Koenigstein, Germany: Koeltz Scientific Books.

Hansen L, Knudsen H. 1992. Nordic Macromycetes Vol. 2 *Polyporales*, *Boletales*, *Agaricales*, *Russulales*. Copenhagen: Nordsvamp.

Hoffmann GF (ed.). 1793. Abbildungen der Schwämme **3**: t. 24.

Horak E. 2005. Röhrlinge und Blätterpilze in Europa. Munich [München]: Spektrum Akademischer Verlag [Elsevier].

Jordan M. 1995. The Encyclopedia of Fungi of Britain and Europe. Newton Abbot, UK: David & Charles.

Lanjouw J, Baehni CH, Robyns W, Rollins RC, Ross R, Rousseau J, Schulze GM, Smith AC, de Vilmorin R, Stafleu FA. 1956. International Code of Botanical Nomenclature. Utrecht, Netherlands: the International Bureau for Plant Taxonomy and Nomenclature of the International Association for Plant Taxonomy.

Mao XL. 2000. The Macrofungi in China. Zhengzhou: Henan Science and Technology Press. (in Chinese).

Massart F. 2003. Le tricholome doré (*Tricholoma auratum* [Fr.] Gillet) au banc des accusés. Documents mycologiques **32**(126): 17–20.

McNeill J, Barrie FR, Burdet HM, Demoulin V, Hawksworth DL, Marhold K, Nicolson DH, Prado J, Silva PC, Skog JE, Wiersema JH, Turland NJ. 2006. International Code of Botanical Nomenclature. Königstein, Germany: Koeltz Scientific Books. (in prep.).

Moser M. 1983. Keys to Agarics and Boleti. London: Roger Phillips.

Nordeloos ME, Christensen M. 1999. 3. *Tricholoma* (Fr.: Fr.) Staude, Mitteldeutschl.: 125. 1857. 107–148. IN Flora Agaricina Neerlandica. Volume 4. C Bas, TW Kuyper, ME Noordeloos, EC Vellinga (eds.). Rotterdam: AA Bakelma.

Persoon CH. 1801. Synopsis Methodica Fungorum. Göttingen, Sweden: Henricum Dieterich.

Phillips R. 1988. Mushrooms and Other Fungi of Great Britain and Europe. London: Pan Macmillan Ltd.

Rogers DP. 1953a. Special committee for fungi, disposition of nomina generica conservanda for fungi. Taxon **2**: 29–32.

Rogers DP. 1953b. Special committee for fungi, disposition of nomina generica conservanda for fungi. Taxon **2**: 102.

Saccardo PA. 1911. Sylloge Fungorum omnium hucusque cognitorum. **20**. Padova.

Singer R. 1962. The *Agaricales* in Modern Taxonomy. 2nd Ed. Weinheim: J. Cramer.

Singer R. 1975. The *Agaricales* in Modern Taxonomy. 3rd Ed. Vaduz: J. Cramer.

Singer R. 1986. The *Agaricales* in Modern Taxononmy. 4th Ed. Koenigstein, Germany: Koeltz Scientific Books.

Stafleu FA, Bonner CEB, McVaugh R, Meikle RD, Rollins RC, Ross R, Schopf JM, Schulze GM, de Vilmorin R. 1972. International Code of Botanical Nomenclature. Utrecht, Netherlands: A. Oosthoek's Uitgeversmaatschappij N.V.

Stafleu FA, Cowan RS. 1979. Taxonomic Literature, 2nd Ed. Volume II: H–Le. Regnum Vegetable **98**: 1–991.

Tai F-L. 1979. Sylloge Fungorum Sinicorum. Beijing: Science Press. (in Chinese).

Tseng S-C. 1996. Fungi of China. Ithaca, New York: Mycotaxon, Ltd.

Tkalčec Z, Mešič A. 2002. Preliminary checklist of *Agaricales* from Croatia. I. Families *Pleurotaceae* and *Tricholomataceae*. Mycotaxon **81**: 113–176.

Voss EG, Burdet HM, Chaloner WG, Demoulin V, Hiepko P, McNeill J, Meikle RD, Nicolson DH, Rollins RC, Silva PC, Greuter W. 1983. International Code of Botanical Nomenclature. Utrecht, Antwerpen: Bohn, Scheltema & Holkema.

Wang X-H, Liu P-G, Yu F-Q. 2004. Color Atlas of Wild Commercial Mushrooms in Yunnan. Kunming: Yunnan Science and Technology Press. (in Chinese).

Wen, H-A, Mao X-L, Sun S-X. 2001. Agarics and other macromycetes. In Higher Fungi of Tropical China. (ed. Zhuang W-Y.), pp 287–351. Ithaca, New York: Mycotaxon Ltd.

Ying J-Z, Zang M. 1994. Economic Macrofungi from Southwestern China. Beijing: Science Press. (in Chinese).

MYCOTAXON

Volume 94, pp. 331–340 October–December 2005

A new species of *Lecanicillium* isolated from the white pine weevil, *Pissodes strobi*

Harry H. Kope & Isabel Leal

Harry.Kope@gov.bc.ca
ileal@pfc.forestry.ca
Natural Resources Canada, Canadian Forest Service
Pacific Forestry Center, 506 West Burnside Road
Victoria, British Columbia, V8Z 1M5, Canada

Abstract—A species of *Lecanicillium* was isolated from the white pine weevil *Pissodes strobi* on Vancouver Island, British Columbia, Canada. Isolates were collected from adult weevil cadavers. The fungus shares some morphological traits with *L. attenuatum* although it differs in having a slower growth rate, larger conidial heads holding up to 50 conidia, a longer average phialide length and variable conidial size and shape. An analysis of the mitochondrial DNA and the β–tubulin gene showed the fungus to be distinct from other *Lecanicillium* species. The new species, *Lecanicillium pissodis*, is described and illustrated.

Key words—entomopathogen, Hyphomycete, *Picea*, Curculionidae, biological control, *Verticillium* section *Prostrata*

Introduction

Numerous entomopathogenic Hyphomycetes have been isolated and described from various insect hosts worldwide (Butt et al. 2001). Entomogenous Hyphomycetes in the genera *Cephalosporium* Corda and *Verticillium* Nees were originally classified by isolates that formed either single conidiophores or verticillate conidiophores, respectively (Petch 1925, 1931, 1932, 1939; Bałazy 1973), and both authors had differentiated multiple taxa within the two genera based on morphological details. In 1971, Gams erected the new section *Prostrata* of the genus *Verticillium* and consolidated a range of *Cephalosporium* isolates that varied greatly in conidial shape and size under one name; *Verticillium lecanii* (Zimm.) Viégas. In 2001, Zare & Gams revisited *Verticillium* sect. *Prostrata* and, by using microscopic and molecular methods, they subsequently placed fifteen species into a new genus, *Lecanicillium* W. Gams & Zare. Their thorough work has resolved many of the difficulties of a broad array of variable isolates of this genus of entomogenous and fungicolous anamorphs of the *Clavicipitaceae*.

In 2003 live adult white pine weevils (*Pissodes strobi* Peck) were collected from Vancouver Island, British Columbia (BC) and while in rearing cages, dead weevils were noted and assayed for the presence of entomopathogenic fungi. The collected fungi were identified as *Lecanicillium muscarium* (Petch) Zare & W. Gams, with the exception of three isolates. Microscopic examination and PCR-RFLP analysis determined that the three unidentified isolates did not fit the descriptions given for known *Lecanicillium* species.

In this paper we report on a new species of *Lecanicillium* that was isolated from cadavers of *P. strobi*. This fungus is represented by three collections. Both the morphological descriptions, restriction fragment length polymorphisms (RFLP) of mitochondrial DNA and the β–tubulin gene, as well as sequencing of the β–tubulin gene indicate they are sufficiently distinctive to warrant a description as a new species of the genus *Lecanicillium*.

Materials and Methods

Isolate collection:

Live weevils were collected from the arboretum at the Pacific Forestry Centre (N48° 28' W124° 24') on Vancouver Island, BC. Weevils were reared on branches of Sitka spruce *Picea sitchensis* (Bong.) Carrière, in cages in a shade-house at the Pacific Forestry Centre. Cadavers were collected, incubated on moistened filter paper in a sealed Petri plate, and observed daily for the development of mycelium. Single spore isolates of *Lecanicillium* were produced by suspending a drop of sterile water from the end of a hypodermic needle, and upon touching a phialide the hydrophilic conidia migrated into the droplet. The droplet was spread over the surface of a *Lecanicillium*-selective medium (LSM) (Kope et al. 2006). Colonies from single germinated conidia were subcultured onto Sabouraud dextrose agar (SDA), grown for 14 days, and then stored at 5°C until needed.

Microscopy:

The identification of *Lecancillium* isolates from colony morphology was assessed on potato-dextrose agar (PDA, Oxoid) after 10 days incubation at 20°C in darkness (Zare and Gams 2001). Conidiophore branching and conidial arrangement was observed in open PDA Petri plates under a compound microscope at 50x magnification. Phialide length and conidial shape and size were observed in water mounts on glass slides under a compound microscope at 400-1000x magnification. Fifty conidia from four water mounts (200 conidia in total) for each of the three isolates were measured to determine conidial size. Photo-micrographs were taken with phase contrast optics. Colony growth of the three *Lecanicillium* isolates was measured on PDA after 10 days incubation at 24°C in darkness.

DNA extraction:

Mycelium for DNA extraction was harvested from potato-dextrose broth by filtration and then freeze-dried. The material was ground to a powder in liquid nitrogen, and the DNA was purified from 20-30 mg of the powdered mycelium using Qiagen DNeasy Plant Mini Kit following the supplied protocol. MtDNA was extracted from 2.0 grams of powdered mycelium using Epicentre's MasterPure™ Yeast DNA Purification Kit, following the supplied protocol to extract total DNA, followed by CsCl/EtBr density gradient equilibrium centrifugation (0.8 g ml^{-1} CsCl and 0.4mg ml^{-1} EtBr) in a Beckman Vti65 rotor at 250,000g for 16 hrs. The band was removed and extracted 3 times with isopropanol and dialyzed against 4L of TE (10mM Tris, 1mM EDTA, pH

8.0) twice for 4 hrs. The resulting DNA was separated into mtDNA and genomic DNA by CsCl/bisbenzimide density gradient centrifugation (1.0 g ml^{-1} CsCl and 0.1 mg ml^{-1} bisbenzimide) in a Beckman Vti65 rotor at 250,000g for 16 hrs. The mtDNA was removed and extracted 3 times with isopropanol and dialyzed against 4L of TE (10mM Tris, 1mM EDTA, PH 8.0) with 3 changes over 16 hrs.

PCR and RLFP:

PCR amplifications and RFLP analyses were carried out as described by Zare and Gams (2001), however, since we had difficulty discerning band patterns using total genomic DNA, we separated the mtDNA from the chromosomal DNA. The RFLP of the ITS region and the 3' partial β–tubulin gene were amplified using the primer sets, ITS–4/ITS–5 (White et al. 1990) and Bt1a/Bt1b (Glass and Donaldson 1995), respectively.

Phylogenetic analysis and sequence alignment:

PCR products of the ITS region and the 5' partial β–tubulin gene were sequenced after purification. To obtain additional β–tubulin sequence information, the 5' partial β–tubulin gene was amplified and sequenced using the primers Bt2E/Bt12 as described by Kim et al. (2003). Sequencing was performed on an ABI 3700 automated sequencer (Perkin-Elmer, Foster City, CA) at the DNA synthesis and sequencing facility, Macrogen (Seoul, Korea). A phylogenetic tree of the ITS region was built using sequences of other taxa obtained from NCBI. The ITS sequences of 16 taxa were aligned using ClustalX (Thompson et al. 1997) and manually adjusted in the PHYDIT program version 3.2 (*http://plaza.snu.ac.kr/~jchun/phydit/*). A phylogenetic tree of relatedness between the new species and related species was constructed by a neighbor-joining (NJ) method (Saitou and Nei 1987). The distances in the ITS region were determined by Kimura's two-parameter model. Branch stability was assessed by 1000 bootstrap replications implemented with PAUP*4.0b10 (Swofford 2001).

Results

Microscopy:

On cadavers of adult *P. strobi*, mycelium can appear as soon as 4 days after infection and it is first seen along the length of the proboscis, around the eyes, at the joins between the thorax, abdomen and head, and at the intersegmented membranes of the legs (Figure 1). The first visible mycelium is white, long and silky, which over time, forms into filamentous mycelium ultimately enveloping the whole cadaver. Shiny globular droplets at the tips of phialides are visible to the unaided eye.

Lecanicillium pissodis can be distinguished from other described species in this genus by a number of morphological characters (Table 1). On agar, colony growth is radial, slightly raised, bright white in colour with a cream to pale yellow reverse and no pigment diffusion into the agar. Prostrate hyphae with phialides are visible at the leading edge of the colony, with phialide numbers increasing towards the center of the colony. Phialides are mostly single with some in a verticillate arrangement of 2-3 at intercalary nodes or at hyphal ends. Conidial heads at the tip of phialides are distinctly visible (Figure 2). When conidial heads are disturbed, conidia are dispersed (Figure 3) releasing up to and sometimes more than 50 conidia. The hydrophilic conidia are quickly spread via water. Conidia are hyaline, single celled, and variable in size, 4–9.2 x 1.6–2.4 μm, and shape, cylindrical to ovoid or oval (FIGURE 4).

TABLE 1. Morphological characteristics of some entomogenous *Lecanicillium* species*

Species	Phialides (μm)	Conidial heads	Conidial size (μm)	Conidial shape
L. aranearum (Petch)Zare & W. Gams	20-30 × 0.7-1.5	—	5.0-8.0 × 1.2-1.5	slightly pointed at one or both ends, straight or curved
L. attenuatum Zare & W. Gams	9-15.5 × 1-2	1-4 conidia in dry clusters	4.5-6.5 × 1.5-2.0	cylindrical, attenuate base uniform shape occasionally 2-celled
L. lecanii (Zimm.) Zare & W. Gams	11-20(30) × 1.3-1.8	globose heads	2.5-3.5(-4.2) × 1.0-1.5	short-ellipsoidal, uniform shape
L. longisporum (Petch)Zare & W. Gams	20-40 × 1.2-2.7	globose heads	5.0-10.5 × 1.5-2.5	ellipsoidal to oblong-oval
L. muscarium	(15-)20-35 × 1-1.7	globose heads	(2-)2.5-5.5(-6) × 1.0-1.5(-1.8)	ellipsoidal or subcylindrical, irregular shape
L. nodulosum (Petch)Zare & W. Gams	10-20 × 1.5	globose heads	2.5-4.5 × 1.2-1.5	oval
L. pissodis sp. nov.	(16-)18-28(-38) × 1-2	globose droplets with up to 50 conidia, or more	4.0-9.2 × 1.6-2.4	cylindrical to ovoid to oval, very variable shape

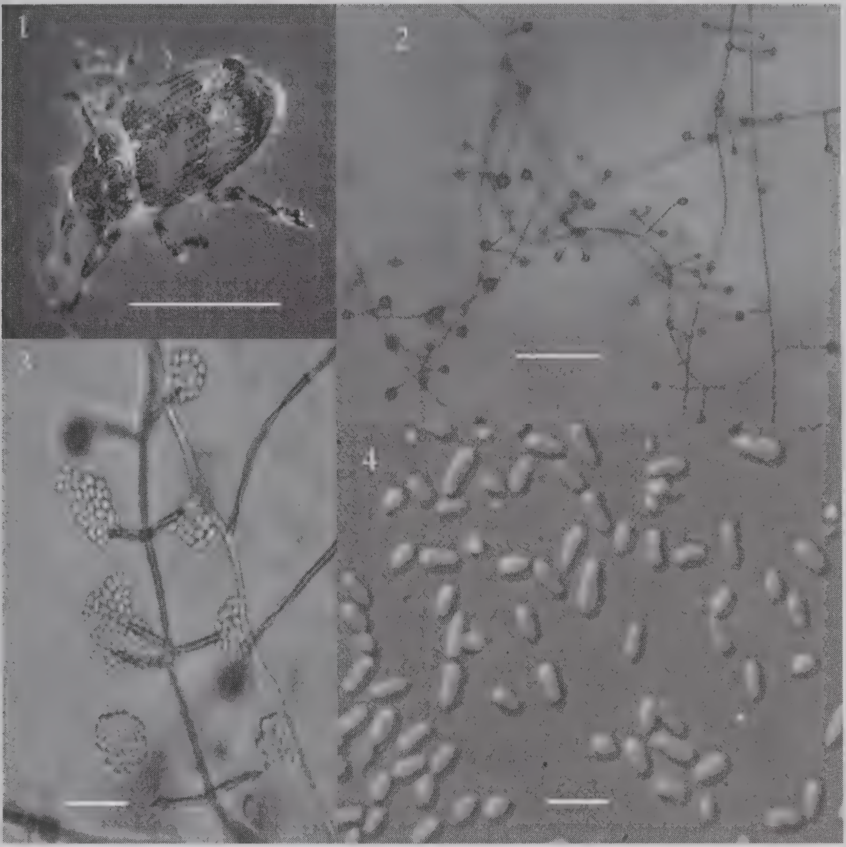
*Characteristics for all species, except *L. pissodis,* taken from Zare and Gams (2001)

Taxonomic Description

Lecanicillium pissodis Kope & I. Leal, **sp. nov.** FIGURES 1-4

> *Coloniae altae, albae, reverso cremeo vel brunnescente, ad 6–7 mm diam. post 10 dies in agaro PDA dicto. Phialides e hyphis prostratus oriundae, singulae vel 3 verticillatae, gradatim in apicem angustatae, 16–(18–28)–38μm x 1–2μm. Conidia in capitulis globosis cohaerentia, ovoidea vel cylindracea, 4–9.2μm x 1.6–2.4μm, magnitudine et forma inaequalia. Crystalla octaedrica copiosa.*
>
> *Species ex Pissodes strobi ad Picea sitchensis in British Columbia Canada*
>
> ***Etymology:*** the specific epithet refers to the genus of the insect host.

Colony growth at 24°C on potato-dextrose agar (Oxoid, PDA) reaches 6–7mm in diameter after 10 days. Colony white, raised, abundant aerial mycelium, reverse cream to pale yellow. Phialides on prostrate hyphae, most as single, some double at right angles and in some instances a verticillate arrangement of up to 3 phialides per node or at the terminal end of hyphae, measuring 16–(18–28)–38μm x 1–2μm and tapered over their length. Conidia, up to and more than 50 formed in globose droplets at the tip of phialides, hyaline, single-celled, cylindrical to oval, to cylindrical with an attenuate end, very variable in size and shape, 4–9.2 x 1.6–2.4μm. Teleomorph unknown. Crystals present in agar medium, octahedral. Growth temperature optimum 20–25°C, with some growth occurring at 5°C and no growth at 30°C.



Figures 1-4. *Lecanicillium pissodis*. 1. Infected adult of *P. strobi*. 2. Prostrate hyphae, phialides with globose droplets holding conidia. 3. Dispersed droplets. 4. Varied shapes and sizes of conidia. Scale: 1 = 5 mm; 2 = 80 µm; 3 = 20 µm; 4 = 10 µm.

Strains examined:

DAVFP 29230, ex *Pissodes strobi* adult, Canada, 2003, H.H.Kope; DAVFP 29231, ex *Pissodes strobi* adult, Canada, 2003, H.H.Kope; DAVFP 29232, ex *Pissodes strobi* adult, Canada, 2003, H.H.Kope, **ex-type**.

Holotype:

DAVFP 29232, isolated from the cadaver of an adult *P. strobi* in BC, Canada, deposited in the Forest Pathology Herbarium, Pacific Forestry Centre, Victoria, BC, Canada. A living ex-type culture CBS 118231 (DAVFP 29232) is deposited in the Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands. This isolate is also preserved at the Fungal Culture Collection of Plant Pests & Diseases Research Institute (Ministry of Agriculture), Tehran, Iran, under IRAN 945 C.

PCR and RFLP:

RFLP patterns of the ITS region, β–tubulin and mitochondrial DNA were compared to other *Lecanicillium* species. No differences in the ITS–RFLP pattern were seen between the three *L. pissodis* isolates and other *Lecanicillium* species. However, clear differences in the RFLP patterns of both the β–tubulin gene and mitochondrial DNA indicated that *L. pissodis* was distinct from other *Lecanicillium* species (Table 2). The β–tubulin gene RFLP-PCR patterns shown in Table 2 for *L. attenuatum* (CBS 170.76) and the three isolates of *L. pissodis* were based on β–tubulin sequencing analysis.

Phylogenetic analysis and sequence alignment:

The alignment of ITS sequences containing the ITS–1–5.8S–ITS region of 12 ITS sequences of other *Lecanicillium* species placed the three isolates of *L. pissodis* in a branch with *L. attenuatum* (Figure 5), which has a high bootstrap value in the NJ tree, and the BLAST search gave 100% identity. Accession numbers for the ITS region of *L. pissodis* 17, 18 and 19 are DQ364668, DQ364669, and DQ364670, respectively.

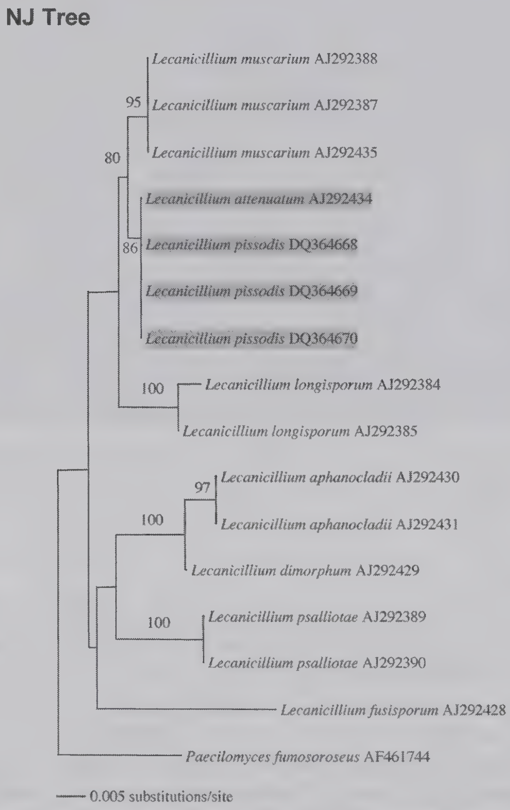

NJ Tree
Lecanicillium muscarium AJ292388
95
Lecanicillium muscarium AJ292387
80
Lecanicillium muscarium AJ292435
Lecanicillium attenuatum AJ292434
86
Lecanicillium pissodis DQ364668
Lecanicillium pissodis DQ364669
Lecanicillium pissodis DQ364670
100
Lecanicillium longisporum AJ292384
Lecanicillium longisporum AJ292385
97
Lecanicillium aphanocladii AJ292430
100
Lecanicillium aphanocladii AJ292431
Lecanicillium dimorphum AJ292429
100
Lecanicillium psalliotae AJ292389
Lecanicillium psalliotae AJ292390
Lecanicillium fusisporum AJ292428
Paecilomyces fumosoroseus AF461744
0.005 substitutions/site

Figure 5. Neighbour joining tree for *Lecanicillium* ITS sequences rooted with *Paecilomyces fumosoroseus* showing the relationship between *L. attenuatum* and *L. pissodis*, and the position of this clade (shaded area) among other *Lecanicillium* species. Numbers on the branches are bootstrap values for major groupings in consensus trees.

Table 2. RFLP-PCR patterns of some taxa of *Lecanicillium* species*

Taxa and accession code	RFLPs of ITS region			RFLPs of the ß–tubulin gene				RFLPs of mt DNA
	Hae III	*Hinf* I	*Msp* I	*Alu* I	*Cfo* I	*Hae* III	*Hinf* I	*Hae* III
L. attenuatum (CBS 170.76)	300, 160, 90, 70	300, 140, 100, 90	290, 140, 110, 60	444, 87	267, 194, 70	353, 178	360, 180	6.2, 5, 4.4, 3
*L. muscarium** (IMI 068689)	300, 160, 90, 70	300, 140, 100, 90	290, 140, 110, 60	450, 90	280, 200	360, 180	540	6.5, 4.4, 3
*L. longisporum** (IMI 021167)	300, 160, 90, 70	300, 140, 100, 90	290, 140, 110, 60	450, 90	280, 200	400, 140	540	6.8, 4, 3.6, 3.5, 3, 2.6
L. pissodis 17 (DAVFP 29230)	300, 160, 90, 70	300, 140, 100, 90	290, 140, 110, 60	444, 87	267, 194, 70	353, 178	225, 181, 119, 6	6.5, 4.2, 3.8, 2.8, 2.3
L. pissodis 18 (DAVFP 29231)	300, 160, 90, 70	300, 140, 100, 90	290, 140, 110, 60	444, 87	267, 194, 70	353, 178	225, 181, 119, 6	6.5, 4.2, 3.8, 2.8, 2.3
L. pissodis 19 (DAVFP 29232)	300, 160, 90, 70	300, 140, 100, 90	290, 140, 110, 60	444, 87	267, 194, 70	353, 178	225, 181, 119, 6	6.5, 4.2, 3.8, 2.8, 2.3

*The shaded area represents the RFLP-PCR fragments for *L. pissodis* in the ß-tubulin gene and mitochondrial DNA

RFLP patterns taken from Zare and Gams (2001)

Sequence alignment of the 3' end of the β–tubulin genes of *L. attenuatum* (CBS 170.76) and *L. pissodis* using primers Bt1a/Bt1b shows only a few sequence differences (Figure 6A). These sequences were deposited at EMBL as DQ364671 for *L. attenuatum* (CBS 170.76) and DQ364672, DQ364673 and DQ364674 for *L. pissodis* 17, 18, and 19, respectively. However, sequence alignment of the 5' end of the β–tubulin gene with primers Bt2E/Bt12 indicated major differences of the exon and intron regions between these two species (Figure 6B). The accession numbers for this part of the β–tubulin gene of *L. attenuatum* (CBS 170.76) is DQ364675, and for *L. pissodis* isolates 17, 18, and 19 are DQ364676, DQ364677, and DQ364678, respectively.

```
A                  1 2 2 2 2 3 3
                   8 0 1 5 8 0 4
                   7 1 6 2 8 9 2
L.attenuatum       T A A C T C T
L.pissodis -17     G G G T C T C
L.pissodis -18     G G G T C T C
L.pissodis -19     G G G T C T C
```

```
B                  0   0
                   1   2
                   8   5
L.attenuatum       T   GTAGCTTTTTAAGGCCCTACCCTGTACGAAATTAGATAGTGGTTCTGAGTATG
L.pissodis -17     C   GTGGTTTCCGACATTCGACTCCGAGACAAGAATTGGTGGATAGCGGTCCTGAC
L.pissodis -18     C   GTGGTTTCCGACATTCGACTCCGAGACAAGAATTGGTGGATAGCGGTCCTGAC
L.pissodis -19     C   GTGGTTTCCGACATTCGACTCCGAGACAAGAATTGGTGGATAGCGGTCCTGAC


                                    1111111111111222 2
                                    0011334455789001 2
                                    1806140967854568 2
L.attenuatum       TTTTGATAG--------CGCCTTTTAGGATGTT GTTTGTTTTACGCACAAACAG
L.pissodis -17     TGTGCTTTTCTCGATAG TATTCCCCTCCGGACC GTTTGTTCTACCCGCGCAACG
L.pissodis -18     TGTGCTTTTCTCGATAG TATTCCCCTCCGGACC GTTTGTTCTACCCGCGCAACG
L.pissodis -19     TGTGCTTTTCTCGATAG TATTCCCCTCCGGACC GTTTGTTCTACCCGCGCAACG


L.attenuatum       CGCGCCTGTCGCGTCGCTAGCAATGTGGTGGATATTAACCATAATTCTCGTACAG
L.pissodis -17     CGTTGCTAGGCTTCGCTGTGGGTACTGACCGCGATTTTGCTCTAG----------
L.pissodis -18     CGTTGCTAGGCTTCGCTGTGGGTACTGACCGCGATTTTGCTCTAG----------
L.pissodis -19     CGTTGCTAGGCTTCGCTGTGGGTACTGACCGCGATTTTGCTCTAG----------


                   33333344444444445555555555555666666666667777
                   46889901245557790112335678899044445677890022
                   83470657370391681348475172614925684658712806
L.attenuatum       TATAATTGCAATTACCCAAGTTCCCTTCAGCCATACCCGATCAT
L.pissodis -17     CTCGTCCTAGTCCGATTTGGCCCTTACCTATTGCCTTTCGCTGG
L.pissodis -18     CTCGTCCTAGTCCGTTTGGCCCTTACCGTATTGCCTTTCGCTGG
L.pissodis -19     CTCGTCCTAGTCCGTTTGGCCCTTACCGTATTGCCTTTCGCTGG
```

Figure 6. Distribution of β–tubulin sequence differences between *L. attenuatum* and *L. pissodis*. **A.** 3' end of β–tubulin gene amplified by primers Bt1a and Bt2a. **B.** 5' end of β-tubulin gene amplified by primers BT2E and BT12. The position of the polymorphism sites in the aligned sequence matrices is written vertically above columns. Bold-typed characters indicate the introns.

Discussion

Lecanicillium pissodis is characteristic of the genus *Lecanicillium* with phialides borne on prostrate hyphae either singly or as multiples arranged in verticillate whorls at nodes, in pairs, or singly. The conidia formed in globose droplets at the end of phialides are single-celled and vary in shape from cylindrical to ovoid or oval.

Morphologically *L. pissodis* is distinct, but closest to *L. attenuatum*. It differs from *L. attenuatum* having a slower growth rate, longer phialides, a greater variability in conidial size and the production of droplets at the end of phialides containing up to 50 conidia. *L. pissodis* differs from *L. lecanii* having a slower growth rate, longer phialides, fewer phialides per whorl, and larger conidia. It also differs from *L. muscarium* and *L. longisporum* by size and conidial shape, respectively and having a slower growth rate than both species.

Lecanicillium pissodis differs in its RFLP banding patterns for both mitochondrial DNA and the β-tubulin gene when compared with other described *Lecanicillium* species. The isolates of *L. pissodis* had ITS sequences identical to that of *L. attenuatum.* The ITS region generally exhibits a high degree of variation between species, and it appears more highly conserved within species. Although, this ribosomal region can be very useful for determining relationships between fungal genera and species (Bruns et al. 1992), it might not allow for the differentiation of closely related taxa (Hermosa et al. 2000, Harrington et al. 2000, Jacobs et al. 2001). To address this, Kim et al. (2004) have suggested that sequences for the β–tubulin gene would be more useful. The sequence differences within the β–tubulin gene reported here separated *L. pissodis* from *L. attenuatum*. This result, coupled with the distinct morphological characteristics, provide strong support for describing *L. pissodis* as a new species.

The genus *Lecanicillium* contains entomogenous fungi with a cosmopolitan distribution occurring on a wide range of insect hosts. *L. muscarium* has been previously collected in British Columbia, Canada (CBS 113450 and 118576) and Kope et al (2000) demonstrated, after fulfilling Koch's postulates, that *L. muscarium* is an entomopathogen of *P. strobi*. The newly described isolate of *L. pissodis*, currently found only in British Columbia, is also pathogenic to *P. strobi* (H. Kope unpublished, fulfilling Koch's postulates). The occurrence and efficacy of *Lecanicillium* in the natural habitat of *P. strobi* would have an effect on weevil populations, making this entomopathogenic fungus a component to be considered in control strategies developed for *P. strobi.*

Acknowledgments

The authors would like to acknowledge the financing supplied by the Enhanced Pest Management Methods, S & T program, to **ECOBIOM** (Extended **Co**llaboration on **B**iological Control of Forest **I**nsects **o**r Pathogenic **M**icroorganisms) under the direction of Dr. Robert Lavallée of the Centre de foresterie des Laurentides, Québec, Canada. We also acknowledge Dr. René Alfaro for additional financing, the technical assistance of Brett Foord with the molecular work, and Dr. Young Lim for his assistance with the PAUP computer program, and Dr. Brenda Callan and Holly Williams for corrections to early versions of the manuscript. We also thank Drs. Richard Humber, Leonard Hutchison, Young Lim and Rasoul Zare for reviewing the manuscript.

Literature cited

Bałazy S. 1973. A review of entomopathogenic species of the genus *Cephalosporium* Corda (*Mycota, Hyphomycetales*). Bulletin de la Société des amis des sciences et des lettres de Poznań. Série D. Sciences biologiques 14: 101–137.

Bruns TD, Vilgalys R, Barns SM, Gonzalez D, Hibbett DS, Lane DJ, Simon L, Stickel S, Szaro TM, Weisberg WG, Sogin ML. 1992. Evolutionary relationships within the fungi: analysis of nuclear small subunit rRNA sequences. Molecular Phylogenetics and Evolution 1: 231–241.

Butt TM, Jackson C, Magan N. 2001. Fungi as biocontrol agents: Progress, problems and potential. New York, CABI Publishing.

Gams W. 1971. *Cephalosporium*-artige Schimmelpilze (Hyphomycetes). Stuttgart, G. Fisher.

Glass L, Donaldson G. 1995. Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous Ascomycetes. Applied and Environmental Microbiology 61: 1323–1330.

Harrington TC, McNew D, Steinel J, Hofstra D, Farrel R. 2001. Phylogeny and taxonomy of the *Ophiostoma piceae* complex and the Dutch elm disease fungi. Mycologia 93: 111–136.

Hermosa MR, Grondona I, Monte E, Iturriaga EA, Diaz-Minguez JM, Castro C, Monte E, Garcia-Acha JM. 2000. Molecular characterization and identification of biocontrol isolates of *Trichoderma* spp. Applied and Environmental Microbiology 66: 1890–1898.

Jacobs K, Wingfield MJ, Wingfield BD. 2001. Phylogenetic relationship in *Leptographium* based on morphological and molecular characters. Canadian Journal of Botany 79: 719–732.

Kim JJ, Kim SH, Lee S, Breuil C. 2003. Distinguishing *Ophiostoma ips* and *Ophiostoma montium*, two bark beetle-associated sapstain fungi. FEMS Microbiology Letters 222: 187–192.

Kim JJ, Lim YW, Wingfield MJ, Breuil C, Kim GH. 2004. *Leptographium bistatum* sp. nov., a new species with a *Sporothrix* synanamorph from *Pinus radiata* in Korea. Mycological Research 108: 699–706.

Kope HH, Alfaro R, Lavallée, R. 2006 (in press). Virulence of the entomopathogenic fungus *Lecanicillium* (Deuteromycota: Hyphomycetes) to *Pissodes strobi* (Coleoptera: Curculionidae). Canadian Entomologist.

Kope HH, Peterson M, Alfaro R. 2000. Pathogenicity of *Verticillium lecanii* to *Pissodes strobi.* Canadian Journal Plant Pathology 22: 187.

Petch T. 1925. Studies in entomogenous fungi. VI: *Cephalosporium* and associated fungi. Transactions of the British Mycological Society 10: 152–182.

Petch T. 1931. Notes on entomogenous fungi. Transactions of the British Mycological Society 16: 55–75.

Petch T. 1932. Notes on entomogenous fungi. Transactions of the British Mycological Society 16: 209–245.

Petch T. 1939. Notes on entomogenous fungi. Transactions of the British Mycological Society 23: 127–148.

Saitou N, Nei M. 1987. The neighbor-joining method: A new method for reconstructing phylogenetic trees. Molecular Biology and Evolution 4: 406–425.

Swofford, DL. 2002. PAUP: Phylogenetic analysis using parsimony and other methods, Version 4.0b10. Sinauer Associates Inc., Sunderland, Massachusetts.

Thompson JD, Gibson T, Plewniak F, Jeanmougin F, Higgins, DG. 1997. The ClustalX Windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Research 24: 4876–4882.

White TJ, Bruns T, Lee S, Taylor J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. 315–322, in MA Innis et al. (eds.), PCR Protocols: a guide to methods and applications. San Diego, Academic Press.

Zare R, Gams W. 2001. A revision of *Verticillium* section *Prostrata*. IV. The genera *Lecanicillium* and *Simplicillium* gen. nov. Nova Hedwigia 73: 1–50.

MYCOTAXON

Volume 94, pp. 341–347 October–December 2005

Two new species of *Hymenochaetaceae* from eastern China

Yu-Cheng Dai

yuchengd@yahoo.com
Institute of Applied Ecology, Chinese Academy of Sciences
Shenyang 110016, China

Bao-Kai Cui

Institute of Applied Ecology, Chinese Academy of Sciences
Shenyang 110016, China
Graduate School, Chinese Academy of Sciences
Beijing 100039, China

Abstract—*Coltricia tsugicola* and *Fomitiporia torreyae* (Basidiomycota, *Hymenochaetaceae*) are described as new from Fujian Province, eastern China. *Coltricia tsugicola* is characterized by small, stipitate and pendent basidiocarps, large and irregular pores and oblong ellipsoid basidiospores; its host tree is *Tsuga chinensis*. *Fomitiporia torreyae* has perennial, resupinate basidiocarps, small basidiospores, no hymenial setae, and grows on *Torreya grandis*.

Key words—polypore, wood-rotting fungi, taxonomy

Introduction

During the study of poroid *Aphyllophorales* from Wuyi Mts. in eastern China, some novel wood-inhabiting fungi were found. Two such specimens were collected on *Tsuga chinensis*: fungi with small, stipitate and pendant basidiocarps, a monomitic hyphal structure without clamp connections, and oblong-ellipsoid, yellowish, smooth, and thick-walled basidiospores. These features fit well with *Coltricia* Gray, but with none of the existing taxa of the genus. Another specimen was collected on living *Torreya grandis*, a gymnosperm tree endemic to eastern China. This collection is a resupinate basidiocarp, a species with dimitic hyphal structure and simple septate generative hyphae. Its basidiospores are subglobose, hyaline, thick-walled, dextrinoid and cyanophilous. These characters indicate *Fomitiporia* Murrill (the *Phellinus robustus* complex), but no suitable species name is available for it. Here we describe these species as new in *Coltricia* and *Fomitiporia*.

Materials and methods

The studied specimens are deposited at the Herbarium of the Institute of Applied Ecology, Chinese Academy of Sciences (IFP). Microscopic features, measurements and drawings were made from slide preparations stained with Cotton Blue and Melzer's reagent. The microscopic procedure fellows Dai (1999). Spores were measured from sections cut from the tubes. KOH = 5% potassium hydroxide, CB = Cotton Blue, CB+ = cyanophilous, CB– = acyanophilous, IKI– = both inamyloid and indextrinoid. In presenting the variation in the size of the spores, 5% of measurements were excluded from each end of the range, and are given in parentheses. In the text the following abbreviations are used: L = mean spore length (arithmetic mean of all spores), W = mean spore width (arithmetic mean of all spores), Q = variation in the L/W ratios between the specimens studied (quotient of the mean spore length and the mean spore width of each specimen), n = number of spores measured from given number of specimens.

Descriptions

Coltricia tsugicola Y.C. Dai & B.K. Cui, **sp. nov.** **Fig.1**

Carpophorum annuum, stipitatum. Facies pororum auratum vel brunneum; pori rotundi vel sinuolati, 1–2 per mm. Systema hypharum monomiticum, hyphae generatoriae septatae, efibulatae, hyphae contexti 4–10 μm in diam. Sporae aureae, oblongo-ellipsoideae vel ellipsoideae, crassitunicatae, IKI–, CB(+), 8.5–11.9 × 5.6–6.9 μm.

Type. — **China.** Fujian Prov., Wuyishan County, Wuyishan Nature Reserve, alt. 1700 m, on rotten wood of *Tsuga chinensis*, 21.X.2005 *Dai 7303* (holotype in IFP, isotype in H).

Etymology. — *Tsugicola* (Lat.): living on *Tsuga*.

Fruitbody. — Basidiocarps annual, centrally stipitate and pendent, solitary or gregarious, soft corky and without odour or taste when fresh, consistency soft corky to fragile and light in weight when dry. Pilei more or less circular or infundibuliform when fresh, becoming shrunken and irregular upon drying, up to 1 cm in diam., 5 mm thick at centre. Pileal surface yellowish to deep reddish brown when fresh, becoming cinnamon or yellowish brown to rust brown upon drying, azonate, velutinate or smooth; margin thin, obtuse, curving down when dry. Pore surface yellowish when fresh, becoming yellowish brown to rust brown upon drying; pores angular or sometimes sinuous to irregular, 1–2 per mm; dissepiments thin, entire. Context cinnamon to rust brown, coriaceous, up to 1 mm thick. Tubes yellowish brown, slightly paler than context, soft corky to slightly brittle when dry, up to 3 mm long. Stipe dark yellowish brown, corky, velutinate, up to 5 mm long, 1 mm in diam.

Hyphal structure. — Hyphal system monomitic; all septa without clamp connections; tissue darkening but otherwise unchanged in KOH.

Context. — Contextual hyphae pale yellowish to golden brown, thin- to fairly thick-walled with a wide lumen, occasionally branched, with frequent simple septa, fairly straight, loosely interwoven, IKI–, CB–, 4–10 μm in diam.; hyphae in the stipe golden yellowish to golden brownish, fairly thick-walled with a wide lumen, occasionally branched, with frequent simple septa, more or less straight, loosely interwoven, IKI–, CB–, 5–9 μm in diam.

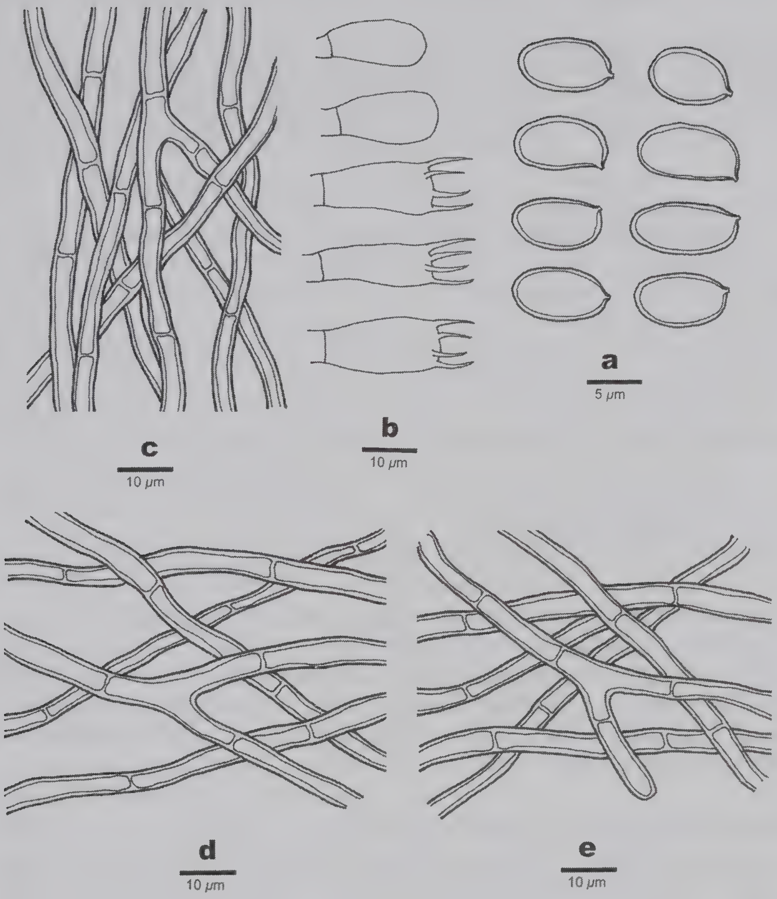


Fig. 1. Microscopic structures of *Coltricia tsugicola* (drawn from the holotype IFP *Dai 7303*). —a: Basidiospores. —b: Basidia and basidioles. —c: Hyphae from tube trama. —d: Hyphae from context. —e: Hyphae from stipe.

Tubes. — Tramal hyphae hyaline to pale yellowish or golden brownish, thin- to slightly thick-walled with a distinct lumen, frequently branched and simple septate, loosely interwoven, IKI–, CB– or very weakly CB+, 4–8 μm in diam. Basidia broadly clavate, with four sterigmata and a simple septum at the base, 14.6–23.8 × 5–8.4 μm; basidioles in shape similar to basidia, but smaller.

Spores. — Basidiospores ellipsoid or more often oblong-ellipsoid, yellowish, thick-walled, smooth, IKI–, CB– or weakly CB+ when juvenile, (8.2–)8.5–11.9(–13.2) × (5.2–)5.6–6.9(–7) μm, L = 9.62 μm, W = 6.31 μm, Q = 1.51–1.54 (*n*=60/2).

Additional specimen (paratype) examined. — **China**, Fujian Prov., Wuyishan County, Wuyishan Nature Reserve, alt. 1700 m, on base of living *Tsuga chinensis*, 21.X.2005 *Dai 7336.*

Remarks. — The species is characterized by small, stipitate and pendant basidiocarps, large and irregular pores, and oblong-ellipsoid basidiospores. *Coltricia tsugicola* was found in a *Tsuga chinensis* forest in the Wuyishan Nature Reserve. Forests of *Tsuga* are restricted to altitudes from 1100 to 1800 meters, close to the timberline which is at ca. 1900 m. Climatically the coniferous forests between these limits vary from warm temperate to almost boreal.

Coltricia focicola (Berk. & M.A. Curtis) Murrill resembles *C. tsugicola* by having oblong-ellipsoid and pale yellowish basidiospores, (7.5–)8–9.5(–10) × (3.8–)4–4.8(–5) µm, $L = 8.55$ µm, W = 4.34 µm, Q = 1.97. However it has larger basidiocarps, lobed or incised at margin, lacerate to dentate dissepiments, and its spores are narrower than in *C. tsugicola*. In addition, *Coltricia focicola* grows on the ground, and it is evidently not a wood-rotting fungus (Ryvarden and Gilbertson 1993).

Coltricia duportii (Pat.) Ryvarden has laterally stipitate basidiocarps and ellipsoid basidiospores, and it may be related to *C. tsugicola.* Its growth habit is not pendent, and fruitbodies are larger, 2.5 cm in diam. and 1 cm thick; besides, its basidiospores are ellipsoid, rusty brown, 8–10 × 6–7 µm. It occurs on deciduous trees in tropical French Guyana and Brazil (Ryvarden 2004).

Basidiospores of the new species are similar to those of *Coltricia montagnei* (Fr.) Murrill which, however, has larger basidiocarps (up to 12 cm in diam. and 1–2 cm thick), poroid to concentrically lamellate hymenophore, and slightly dextrinoid basidiospores (Núñez and Ryvarden 2000, Ryvarden and Gilbertson 1993).

The minute, stipitate and pendant basidiocarps make *Coltricia tsugicola* confusingly similar to *Coltriciella dependens* (Berk. & M.A. Curtis) Murrill, but spores of *C. dependens* are finely verrucose, and it occurs in the tropics (Gilbertson and Ryvarden 1986, Ryvarden and Johansen 1980).

Fomitiporia torreyae Y.C. Dai & B.K. Cui, **sp. nov.** **Fig.2**

Carpophorum perenne, resupinatum, contextum atroumbrinum vel brunneum. Facies pororum ravide umbrina vel umbrina; pori rotundi vel sinuolati, 4–6 per mm. Systema hypharum dimiticum, hyphae generatoriae septatae, efibulatae. Sporae subglobosae vel globosae, crassitunicatae, dextrinoidae, CB+, 5–5.9 × 4.4–5.3 µm.

Type. — **China.** Fujian Prov., Wuyishan County, Wuyishan Nature Reserve, alt. 800 m, on living *Torreya grandis*, 21.X.2005 *Dai 7320* (holotype in IFP, isotype in H).

Etymology. — *Torreyae* (Lat.): referring to the host tree genus.

Fruitbody. — Basidiocarps perennial, resupinate, inseparable, woody hard when fresh, without odour or taste, consistency woody hard and light in weight when dry, up to 20 cm or more in longest dimension, 10 cm wide, ca. 8 mm thick at centre; margin more or less receding, up to 2 mm wide, pale brown. Pore surface greyish brown when fresh, becoming pale brown to rust brown upon drying, shining, slightly cracked with age; pores circular to sinuous at oblique surface, 4–6 per mm; dissepiments thin, entire. Subiculum umber brown, woody hard, very thin, up to 0.5 mm thick. Tubes yellowish brown to rust brown, hard corky, up to 7 mm long, distinctly stratified.

Hyphal structure. — Hyphal system dimitic; all septa without clamp connections; skeletal hyphae IKI–, CB–; tissue darkening but otherwise unchanged in KOH.

Subiculum. — Tissue dominated by skeletal hyphae; generative hyphae hyaline to pale yellowish, thin- to fairly thick-walled, occasionally branched and frequently simple septate, 2–3.2 µm in diam.; skeletal hyphae golden brown to rust brown, thick-walled with a narrow to wide lumen, unbranched, interwoven, more or less agglutinated, 2.3–4.5 µm in diam.

Tubes. — Tramal hyphae dominated by skeletal hyphae; generative hyphae hyaline, thin- to slightly thick-walled, occasionally branched and frequently simple septate, 1.8–3 µm in diam.; skeletal hyphae golden brown to rust brown, thick-walled with a narrow to wide lumen, unbranched, interwoven, slightly agglutinated, 2–4.2 µm in diam. Subhymenium indistinct. Hymenial setae absent, setae extremely rare in subiculum, subulate to ventricose, dark brown, thick-walled, 10–16 × 6–7 µm; cystidioles frequent, subulate, sharp-pointed or obtuse at apex, hyaline, thin-walled, 14–19.7 × 2.8–4 µm; basidia barrel-shaped to subglobose, with four sterigmata and a simple septum at the base, 8–13 × 6.8–9 µm; basidioles in shape similar to basidia, but slightly smaller; big rhomboid crystals abundant in hymenia and trama.

Spores. — Basidiospores subglobose to globose, hyaline, thick-walled, smooth, dextrinoid in Melzer´s reagent, strongly CB+, (4.5–)5–5.9(–6) × (4–)4.4–5.3(–5.9) µm, L = 5.46 µm, W = 4.90 µm, Q = 1.11 (*n*=60/1).

Remarks. — The perennial growth habit, resupinate basidiocarp, lack of setae, presence of subulate cystidioles, small basidiospores, and growth on *Torreya grandis* characterize this species. We could not find hymenial setae in the type, and only a few setae were found in the subiculum and subhymenium. Even there they are extremely rare: 4 setae were observed from 5 microscope preparations. *Fomitiporia torreyae* is the third resupinate species in the genus in China, the other two being *F. punctata* (P. Karst.) Murrill and *F. bannaensis* Y.C. Dai (Dai 1999, Dai *et al.* 2001).

Four species in *Fomitiporia*—*F. aethiopica* Decock et al., *F. mediterranea* M. Fisch., *F. punctata* and *F. tabaquilio* (Urcelay et al.) Decock & Robledo—have resupinate basidiocarps, and lack hymenial setae (Decock *et al.* 2005). These species have distinctly larger basidiospores than *F. torreyae*, the length being more than 6 µm.

Fomitiporia tenuis Decock et al. was recently described from Africa (Decock *et al.* 2005). Its basidiospores are very similar to those of *Fomitiporia torreyae*. However, the former has a thinner fruitbody (up to 2 mm thick), smaller pores (10–11 per mm), and hymenial setae.

Fomitiporia bannaensis is found in China too but it is distinguished from *F. torreyae* by smaller pores (8–10 per mm) and abundant hymenial setae. Furthermore, *F. bannaensis* is a subtropical species, growing on angiosperm trees (Dai *et al.* 2001).

Besides the above-mentioned taxa, the remaining species of *Fomitiporia*, *F. sonorae* (Gilb.) Y.C. Dai, *F. pseudopunctata* (A. David et al.) Fiasson, *F. sublaevigata* (Cleland & Rodway) Y.C. Dai, have principally resupinate fruitbodies, but all have hymenial setae and grow on angiosperm trees. *Fomitiporia sonorae* has resupinate to reflexed basidiocarps, and its setae are long (35–55 × 5–8 µm, Gilbertson 1979, Valenzuela &

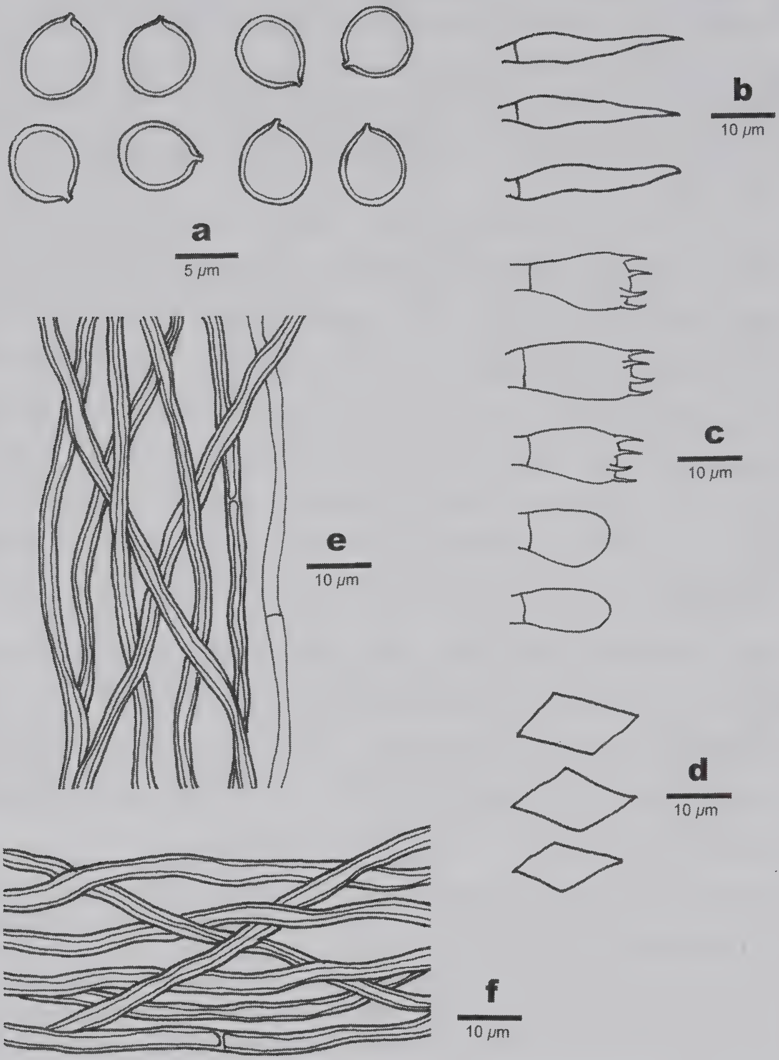


Fig. 2. Microscopic structures of *Fomitiporia torreyae* (drawn from the holotype IFP *Dai 7320*). —a: Basidiospores. —b: Cystidioles. —c: Basidia and basidioles. —d: Rhomboid crystals. —e: Hyphae from tube trama. —f: Hyphae from subiculum.

Chacón-Jiménez 1991). *Fomitiporia pseudopunctata* and *F. sublaevigata* have bigger spores (6.5–7.5 × 5.5–7 μm in *F. pseudopunctata*, 6.5–7 × 5–6 μm in *F. sublaevigata*, Decock et al. 2005).

Acknowledgements

Special thanks are due to Mr. Mingyun Huang for companionship during the field trip. We express our gratitude to Prof. Teuvo Ahti (H, Finland) for revising the Latin description and improving the manuscript, and to Drs. Tuomo Niemelä (University of Helsinki, Finland), and Peter K. Buchanan (Landcare Research, New Zealand) who reviewed the manuscript. The research was financed by the National Natural Science Foundation of China (Project No. 30425042).

Literature cited

Dai YC. 1999: *Phellinus sensu lato* (*Aphyllophorales, Hymenochaetaceae*) in East Asia. Acta Bot. Fennica 166: 1–115.

Dai YC, Zhang XQ, Zhou TX. 2001: New and noteworthy species of *Hymenochataceae* from China. Mycosystema 20: 16–21.

Decock C, Bitew A, Castillo G. 2005: *Fomitiporia tenuis* and *Fomitiporia aethiopica* (Basidiomycetes, *Hymenochaetales*), two undescribed species from the Ethiopian highlands—taxonomy and phylogeny. Mycologia 97: 121–129.

Gilbertson RL, 1979: The genus *Phellinus* (*Aphyllophorales, Hymenochaetaceae*) in western North America. Mycotaxon 9: 51–89.

Gilbertson RL, Ryvarden L. 1986: North American polypores 1. *Abortiporus – Lindtneria.* Fungiflora, Oslo. 433 pp.

Núñez M, Ryvarden L. 2000: East Asian polypores 1. *Ganodermataceae* and *Hymeno-chaetaceae.* Syn. Fungorum 13: 1–168.

Ryvarden L. 2004: Neotropical polypores 1. Syn. Fungorum 19: 1–229.

Ryvarden L, Gilbertson RL. 1993: European polypores 1. Syn. Fungorum 6: 1–387.

Ryvarden L, Johansen I. 1980: A preliminary polypore flora of East Africa. Fungiflora, Oslo. 636 pp.

Valenzuela R, Chacón-Jiménez S. 1991: Los poliporaceos de Mexico 3. Algunas especies de la Reserva de la Biosfera el Cielo, Tamaulipas. Revista Mex. Micol. 7: 39–70.

MYCOTAXON

Volume 94, pp. 349–355 October–December 2005

Some entomogenous fungi from Wuyishan and Zhangjiajie Nature Reserves

2. Three new species of the genus *Hirsutella*[*]

ZONGQI LIANG [1*] YANFENG HAN[1]
AIYING LIU[1] & JIANZHONG HUANG[2]

*zqliang472@yahoo.com.cn**
swallow112886@yahoo.com.cn ayliu6@yahoo.com.cn
[1] The Institute of Fungus Resources, Guizhou University
Guiyang 550025, China

hjz@fjnu.edu.cn
[2] The Key Laboratory of Microbiological Engineering
Fujian Normal University, Fuzhou 350007, China

Abstract—Three new entomogenous fungi of the genus *Hirsutella, H. zhangjiajiensis, H. hunanensis* and *H. crinita,* collected from Wuyishan (Wuyi Mountains) and Zhangjiajie Nature Reserves, are described and illustrated. Some problems of the identification of these fungi are briefly discussed.

Key Words—hyphomycete, taxonomy, morphology

Introduction

Hirsutella Pat. is one of the most abundant and important entomogenous fungi. In 1990, we reviewed the development, taxonomic characteristics of the genus, and proposed a key to 62 taxa. A few doubtful species were discussed briefly (Liang 1990a, b). Seifert & Boulay (2004) suggested that *Hirsutella* consists of 65 species. From our study, we refer 90 species around the world to the genus of *Hirsutella.*

The genus *Hirsutella* plays an important role in the natural control of pest insects (Evans 1974, 1982). *H. gigantea* Petch can infect many larvae and pupae of Lepidoptera in the Kuankuoshui Preserve in Guizhou, China (Liang 1991a). Besides this fungus, *H. rhossiliensis* Minter & B. L. Brady, discovered in the early 1980's, has a stronger lethiferous effect on many plant parasitic nematodes, such as *Ditylenchus dipsaci, Meloidogyne incognita, Aphelenchoides fragariae* and *Criconemella xenoplax* (Jaffee et al. 1982; Cayrol & Frankowski 1986; Cayrol et al. 1986).

It is known that many species in the genus *Hirsutella* are anamorphs of *Cordyceps* Fr. For example, an anamorph of the famous Chinese traditional medicine *C. sinensis* (Berk.) Sacc. is *H. sinensis* (Liu et al., 1989). Some valuable bioactive compounds have been recently discovered from members of *Hirsutella.* A protein toxic to insects, hirsutellin

---

[*] Corresponding author: Zongqi Liang

A (HtA), has been purified from the fungal mite pathogen, *H. thompsonii* F. E. Fisher (Mazet & Vey, 1995). HtA is considered to be one of many ribonuclease inactivator proteins (RIPs) (Maimala et al. 2002). Vongvanich et al.(2002) found hirsutellide A from a new species of *Hirsutella*, an interesting antimycobacterial cyclohexadepsipeptide. The synthesis of its key precursor has been studied (Xu et al. 2005).

From 1989 to 2001, Chinese mycologists reported seven new species of *Hirsutella* (Table 1). The present paper describes three additional new species of *Hirsutella* and their taxonomic position within the genus.

Table 1. Previously reported species of *Hirsutella* in China

Fungal Name	References
H. leizhouensis H.M. Fang & S.M. Tan	Fang & Tan 1992
H. changbeishanensis Z.Q. Liang	Liang 1991b
H. polycolluta Z.Q. Liang	Liang 1991b
H. yunnanensis Z.Q. Liang & A.Y. Liu	Liu et al., 1993
H. yunnanensis var. *tenuisynnemata* Z.Q. Liang & A.Y. Liu	Liang et al.,1997
H. sinensis X.J. Liu et al.	Liu et al., 1989
H. longissima C.R. Li et al.	Li et al., 1999
H. huangshanensis C.R. Li et al.	Li et al., 2005

Materials and Methods

All collected specimens were routinely oven-dried at 40C to prevent growth of contaminant fungi. For examination by light microscopy, slide preparations were made of a snippet of outer layer tissue from the delaminated synnemata by mounting in lactophenol and cotton blue.

Description of new species

Hirsutella zhangjiajiensis Z.Q. Liang & A.Y. Liu **sp. nov.** **Figs. 1-1, 4**

> *Stromatibus solitariis vel binariis, cylindraceis, simplicibus, 100 × 2 mm. Stipite et capitulo brunneo vel ochraceo. Phialides e ascosporis exorentibus, subulatiae graciles, 30-52 × 2-4.5 μm, vel basi inflata ellipsoida, 4.5 × 3μm. Conidia lanceolata or leviter curvata, 4.5-7.5 (-10) × 1.5-2.5 μm, mucigeri, lemoniformes, 10 × 4 μm. Holotypus: GZDXIFR98-7131.*

Stroma single or 2, cylindrical, 100 × 2 mm, not ramified, leathery. Stipe and fertile part brown to snuff-colored. Conidiogenous structure deriving from the microcycle conidiation of ascospores from *Cordyceps zhangjiajiensis*. Phialides slender awl-shaped, 30-52 × 2-4.5 μm or inflated ellipsoidal at basal portion, 4.5 × 3 μm. Conidia lanceolate or the shape of an orange segment, 4.5-7.5(10) × 1.5-2.5 μm, embedded in a mucous sheath, limoniform, 10 × 4 μm.

Specimen studied: GZDXIFR98-7131 was collected from Zhangjiajie Nature Reserve, Hunan Province by LIANG Zongqi, LIU Aiying et al. in VII 1998.

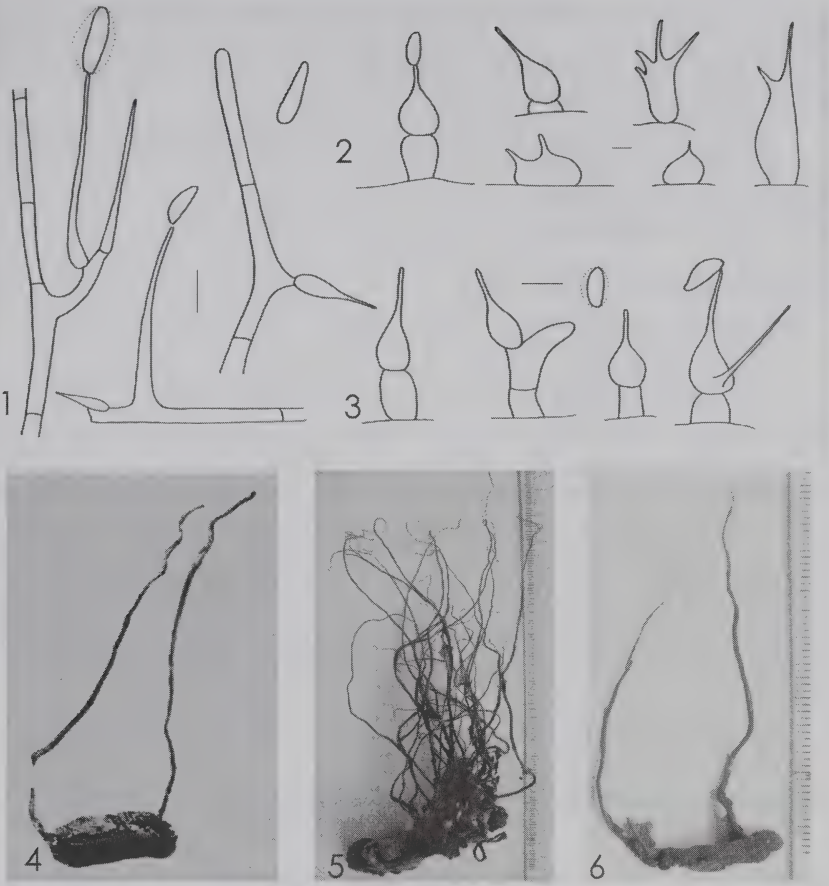


Fig. 1 Synnemata and conidiogenous structures of three species of *Hirsutella*. 1-1, 4: Conidiogenous structures and synnemata of *H. zhangjiajiensis*. 1-2, 5: Conidiogenous structures and synnemata of *H. crinta*. 1-3, 6: Conidiogenous structures and synnemata of *H. hunanensis*

Bars1, 2 =10μm, Bar3 =5μm

Habitat: Pupa of Lepidoptera.
Teleomorph: *Cordyceps zhangjiajiensis* Z.Q. Liang & A.Y. Liu (Liang et al. 2002).
Distribution—Zhangjiajie Nature Reserve: Hunan Province, China.

Hirsutella hunanensis Z.Q. Liang **sp. nov.** **Figs. 1-3, 6**

> *Synnemata erecta, filiformes, simplices, 5-55 mm longa, 1mm crassa, flexiles vel ligneae, nigro-brunneae. Phialides 7.5-9 × 4.5-5 μm, e basi inflate cylindrica vel pyriformibus in cillum 0.5-0.8 μm crassum angustatae, prolificis. Conidia ellipsoidea vel leviter curvata, 6 × 1.5-2 μm, mucigeri, ellipsoideis, 6 × 4.5 μm. Holotypus: GZDXIFR98-7132*

Synnemata filiform, erect, unbranched, 5-55 mm long, 1mm wide, flexible to ligneous, dark brown, arising from between head and thorax parts of host insect. Phialides solitary or crowded along synnemata, mostly with cylindrical or pyriform inflated basal portion, 7.5-9 × 4.5-5 μm and with one or 2 slender, thin necks, 7.5-11 × 0.5-0.8 μm. Conidia long ellipsoidal to the shape of an orange segment, 6 × 1.5-2 μm; embedded in a mucous sheath, ellipsoidal, 6 × 4.5 μm. Teleomorph not observed.

> Specimen studied: GZDXIFR98-7132. was collected from Zhangjiajie Nature Reserve, Hunan Province by LIANG Zongqi, LIU Aiying et al. in VII 1998.

Habitat: A larva of Lepidoptera.
Distribution: Zhangjiajie Nature Reserve—Hunan Province, China.

Hirsutella crinita Z.Q. Liang **sp. nov.** **Figs. 1-2, 5**

> *Synnemata erecta, filiformes vel cylindrica, simplices, 170 mm longa, 1-1.5 mm crassa, flexiles vel ligneae, nigro-brunneae, caespitosa. Phialides 4.5-6(-10) × 3.5-4.5 μm, e basi inflata globosa vel cylindrical in collum 0.3-0.5 μm crassum angustatae, prolificis. Conidia rara, clavata angusta, 6-9 × 1-1.5 μm, exmuca. Holotypus: GZDXIFR98-5231.*

Synnemata slender cylindrical or filiform, unbranched, flexible to ligneous, dark brown, caespitose, arising from the head and thorax parts of host insect, very long (up to 170 mm), slightly swollen in the basal region, 1.0-1.5mm wide, in middle part to upside, 0.5-1.0 mm wide. Phialides somewhat scattered, forming a loose hymenium, arising as lateral cells from the outer hyphae of the synnemata, hemispheric to globose or cylindrical, inflated at basal portion, 4.5-6(-10) × 3.5-4.5 μm, abruptly narrowing into a short thin neck, 1.5-4 μm long and 0.3-0.5 μm wide, always proliferating 2-4, sometimes forming inflated, sterile cylindrical hyphae, 15 × 4.5-5 μm. Conidia infrequent, narrowly anisomerous obclavate, 6-9 × 1-1.5 μm, absence of distinct mucus layer. Teleomorph not observed.

> Specimen studied: GZDXIFR98-5231 was collected from Wuyishan, Hujian Province by LIANG Zongqi, LIU Aiying et al. in V 1998.

Habitat: A larva of Lepidoptera. 80-100 × 50-80 mm.
Distribution: Wuyishan, Fujian Province, China.

Discussion

Evans & Samson (1982a,b, 1984) reported several species with phialides of two types (Table 2): A-phialides, which are lateral and compacted in a layer below the head, and B-phialides, which are terminal, compact, and awl-shaped. The new species, *H. zhangjiajiensis,* forms both A- and B-phialides during the ascosporic microcycle conidiation (Fig. 1-1) and produces B-phialides that are solitary and compacted. Species in the genus *Hirsutella* that have awl-shaped phialides more than 40 μm long include *H. aphidis*, *H. stilbelliformis* var. *stilbelliformis*, *H. stilbelliformis* var. *dolichoderi*, *H. sporodochialis*, *H. darwinii*, *H. guignardii*, *H. sinensis*, and *H. zhangjiajiensis*. Among them, species that have both A- and B-phialides and some phialides greater than 40μm long are *H. sporodochialis*, *H. stilbelliformis* var. *stilbelliformis*, *H. stilbelliformis* var. *dolichoderi* and *H. zhangjiajiensis* (Table 2). Possession of rough-walled hyphae and echinate phialides separates the three former species from *H. zhangjiajiensis*.

In the genus *Hirsutella*, some species that have proliferating phialides are *H. besseyi*, *H. guyana*, *H. versicola*, *H. verticillioides*, and *H. yunnanensis* var. *tenuisynnemata*. All

Table 2. A comparison of three new *Hirsutella* with related species

Species	Phialides			Conidia (µm)	Reference
	Shape	Length in µm (max.)	Type		
H. aphidis Petch	Awl	>40	B	Cymbiform 9×1.5-2.5	Petch 1942
H. besseyi F.E. Fisher	Awl or cylindrical	>40	B	Ellipsoid or limoniform 4.1-8.3×2.5-5.8	Minter & Brady 1980
**H. crinita*	Base inflated: subglobose	>10	A	Narrowly clavate, 6-9 × 1-1.5	This work
H. darwinii H.C. Evans & Samson	Awl	>40	B	Fusiform 4.5-11.5×1.5-2	Evans & Samson 1982a
H. guignardii (Maheu) Samson et al.	Awl	>40	B	Ellipsoid or fusiform 7-13×4-6	Samson et al. 1984
H. guyana Minter & B.L. Brady	Base inflated: cylindric	>40	A	Ellipsoid or an orange segment 8-12×3-7	Minter & Brady 1980
**H. hunanensis*	Base inflated: cylindric to pyriform	>10	A	Ellipsoid or an orange segment 6 × 1.5-2	This work
H. minnesotensis S.Y. Chen et al.	Base inflated: subglobose	>10	A	Globose 4-6 (in length)	Chen et al. 2000
H. necatrix Minter et al.	Base inflated: subglobose & awl	>5 >10	A & B	Ovoid or ellipsoid 3-4×2.5-3 Globose, -3.5	Minter et al. 1983
H. sinensis Liu et al.,	Awl	>40	B	Reniform or ellipsoid 5.4.-14×3.2-5.4	Liu et al., 1989
H. sporodochialis H.C. Evans & Samson	Base inflated: flask-shaped or awl	>80	A to B	Fusiform 10-27×3.5-4	Evans & Samson 1984
H. stilbelliformis H.C. Evans & Samson	Base inflated: ellipsoid echinate & awl	>10 >100	A & B	Clavate 7-9×1.5-2.5 Ovoid 8-12×4-5	Evans & Samson, 1982b
H. stilbelliformis var. *dolichoderi* H.C. Evans & Samson	Base inflated: ellipsoid echinate & awl	>40 >100	A & B	Cylindrical to ovoid 6.5-9.5×3.5-4.5	Evans & Samson, 1982b
H. versicola Petch	Base inflated: ellipsoid	>40	A	Fusiform 4-7×1	Minter & Brady 1980
H. verticillioides Charles	Base inflated: cylindrical	>40	A	Ellipsoid or an orange segment 6-8×3-5	Minter & Brady 1980
H. yunnanensis var. *tenuisynnemata* Z.Q. Liang & A.Y. Liu	Base inflated: ellipsoid or subglobose	>10	A	Cylindrical or obclavate 4.5-7.2×1.5-1.8	Liang et al.,1997
**H. zhangjiajiensis*	Awl & base inflated: ellipsoid	>40	A & B	Lanceolate or an orange segment, 4.5-10× 1.5-2.5	This work

of these species are similar to the new species *H. hunanensis* in fusiform, ellipsoidal and orange conidia. *H. hunanensis* can be distinguished from the above-mentioned species by phialides less than 10µm length and smaller conidia, around 6 × 1.5-2 µm.

Minter et al. (1983) described a new species, *H. necatrix*, that also has phialides with a subglobose or globose swollen base portion. Possession of ovate or ellipsoidal separates this fungus from *H. crinita*, which has narrowly clavate conidia (Table 2). The new species *H. crinita* is also closely related to *H. minnesotensis* and *H. yunnanensis* var. *tenuisynnemata* with similar phialides containing a subglobose basal portion. Globose conidia typically found in *H. minnesotensis* distinguish it from *H. crinita. H. yunnanensis* var. *tenuisynnemata* has fusiform or ellipsoidal conidia and can produce yellow caespitose synnemata.

Acknowledgments

This project was supported by the National Natural Science Foundation of China. We are grateful to Drs. Shengjun Chang and Jielin Liu for their comments on the manuscript. And we really appreciate editorial revisions.

References

Cayrol JC, Frankowski JP. 1986. Influence of the number of parasitizing conidia of *Hirsutella rhossiliensis* on the mortality of *Ditylenchus dipsaci*. Revue Nématol. 9(4): 411-412.

Cayrol JC, Castet R, Samson RA. 1986. Comparative activity of different *Hirsutella* species towards three plant parasitic nematodes. Revue Nématol. 9(4): 412-414.

Chen SY, Liu XZ, Chen FJ. 2000. *Hirsutella minnesotensis* sp. nov., a new pathogen of the soybean cyst nematode. Mycologia 92(5): 819-824.

Evans HC, Samson RA. 1982a. Entomogenous fungi from the Galápagos Islands. Can. J. Bot. 60: 2325-2333.

Evans HC, Samson RA. 1982b. *Cordyceps* species and their anamorphs pathogenic on ants (Formicidae) in tropical forest ecosystems II. The *Camponotus* (*Formicinae*) complex. Trans. Br. Mycol. Soc. 82 (1): 127-150.

Evans HC, Samson RA. 1984. *Cordyceps* species and their anamorphs pathogenic on ants (Formicidae) in tropical forest ecosystems I. The *Cephalotes* (*Myrmicinae*) complex. Trans. Br. Mycol. Soc. 79(3): 431-453.

Evans HC. 1974. Natural control of arthropods with special reference to ants by fungi in the tropical high forest of Ghana. J. Appl. Ecology (11): 37-49.

Evans HC.1982. Entomogenous fungi in tropical forest ecosystem: An appraisal. Ecological Entomology (7): 47-60.

Fang HM, Tan SM. 1992. A new species of *Hirsutella*, *H. leizhouensis* Fang & Tan. Acta Mycologica Sinica 11(1): 28-31.

Jaffee BA, Zehr EL. 1982. Parasitism of the nematode *Criconemella xenoplax* by the fungus *Hirsutella rhossiliensis*. Phytopathology 72: 1378-1381.

Li CR, Huang B, Fan MZ, Li ZZ. 1999. *Cordyceps longissima* and its *Hirsutella* anamorph. Proceedings of 4th Sino-Korean Joint Symposium on Mycology. J. Anhui Agri. Univ. 26(3): 374-377.

Li CR, Xia CR, Lin YR, Fan MZ & Li ZZ. 2005. *Hirsutella huangshanensis* sp.nov., the anamorph of *Cordyceps formosana* and its infection on *Tenebrio molitor* Mycosystema 24(3): 349-355.

Liang ZQ. 1990a. Classification of the genus *Hirsutella* Pat. I. Advance and the characteristics of taxonomy. Journal of Guizhou Agricultural College 9 (1): 58-68.

Liang ZQ. 1990b. Classification of the genus *Hirsutella* Pat. II. Key to the taxa of the genus *Hirsutella* Pat. Southwest China Journal of Agricultural Sciences 3 (3): 32-39.

Liang ZQ. 1991a. Several entomopathogenic new species and records of Stilbelaceae in China. Journal of Guizhou Agricultural College 10 (1): 56-62.

Liang ZQ. 1991b. Two new entomopathogenic fungi of the genus *Hirsutella*. Southwest China Journal of Agricultural Sciences 4 (1): 51-56.

Liang ZQ, Liu AY & Huang JZ. 2002. Some entomogenous fungi from Wuyishan and Zhangjiajie Natural Reserves I. *Cordyceps* spp. *Mycosystema* 21(2): 162-166.

Liang ZQ, Liu AY, Huang JZ & Jiao YC. 1997. The genus and its allies from Kuankuoshui Preserve in Guizhou II. Mycosystema 16 (1): 61-67.

Liu AY, Liang ZQ, Cao L & Yang HM. 1993. A few new and rare entomogenous fungi in Kunming area. Southwest China Journal of Agricultural Sciences 6 (1): 87-90.

Liu XJ, Guo YL, Yu YX & Zeng W. 1989. Isolation and identification of the anamorphic state of *Cordyceps sinensis* (Berk.) Sacc. Acta Mycologica Sinica 8(1): 35-40.

Maimala S, Tartar A, Boucias D & Chandrapatyab A. 2002. Detection of the toxin Hirsutellin A from *Hirsutella thompsonii*. Journal of Invertebrate Pathology 80: 112–126.

Mazet I & Vey A. 1995. Hirsutellin A, a toxic protein produced in vitro by *Hirsutella thompsonii*. Microbiology 141: 1343-1348.

Minter DW, Brady BL. & Hall RA. 1983. Five hyphomycetes isolated from eriophyid mites. Trans. Br. Mycol. Soc. 81(3): 455-471.

Petch T. 1942. Notes on entomogenous fungi. Trans. Br. Mycol. Soc. 25: 259-260.

Samson RA, Rombach MC & Seifert K. 1984. *Hirsutella guignardii* and *Stilbella kervillei*, two troglobiotic entomogenous hyphomycetes. Persoonia 12(2): 123-134.

Seifert KA & Boulay H. 2004. *Hirsutella uncinata*, a new hyphomycete from Australia. Mycologia 96(4): 929-934.

Vongvanich N, Kittakoop P, Iaska M, Trakulnaleamsai S, Vimuttipong S, Tanticharoen M & Thebtaranonth Y. 2002. Hirsutellide A, a new antimycobacterial cyclohexadepsi peptide from the entomopathogenic fungus *Hirsutella kobayasii*. J. Nat. Prod. 65(9): 1346-1348.

Xu YJ, Duan XM, Li ML, Jiang LQ, Zhao GL, Meng Y & Chen LG. 2005. Synthesis of the key precursor of Hirsutellide A. Molecules 10: 259-264.

MYCOTAXON

Volume 94, pp. 357–363 October–December 2005

Paecilomyces parvosporus, a new species with its relatives from Yunnan Province, China

YANFENG HAN[1] ZONGQI LIANG[1]
HUALI CHU[2] & JICHUAN KANG[3]

[1]swallow112886@yahoo.com.cn *zqliang472@yahoo.com.cn
Institute of Fungus Resources, Guizhou University
Guiyang, China 550025

[2]huali1121@163.com
Hainan University, Haikou, 570228 China

[3] Jichuank@yahoo.co.uk
The Research Center of Biochemistry Engineering
Guizhou University Guiyang, China 550025

Abstract—Four *Paecilomyces* isolates were isolated from soils and insects infected in Yunnan Province, China. Amongst them, GZDX-IFR-A10.1 has been distinguished as a new species by its unique morphological characters including a light yellow colony on Czapek, small subglobose or globose conidia and long and thin phialidic necks as well as a phylogenetic analysis based on the nucleotide sequences of the ITS region. Meanwhile the other three isolates GZDX-IFR-A35.1, GZDX-IFR-466.6 and GZDX-IFR-468.2 were identified as *P. lilacinus, P. tenuipes* and *P. cateniannulatus.*

Key Words—taxonomy, morphology, fungi, rDNA sequence

Introduction

China is rich in fungal species in *Paecilomyces*. Recently we have found several new species in *Paecilomyces* (Han et al. 2005a, b, Liang et al. 2005). In 2004, while carrying out a project of the National Natural Science Foundation of China (NSFC) to investigate the *Paecilomyces* species in Kunming, Nujiang, Lancangjiang Xishuangbanna (Tropical rain forest), Tengchong (Geothermal National Geopark) and Lijiang areas in Yunnan province, we collected many *Paecilomyces* samples from the soils and infected insects. Amongst them, quite a few species in *Paecilomyces* known in China, including *P. cateniannulatus, P. tenuipes, P. farinosus, P. catenioblíquus* (Liang 1981) and *P. amoeneroseus* (Liu & Liang 2003) were collected and isolated again. Moreover, a few *Paecilomyces* members with mesophilic and thermotolerant nature from soils have also been obtained (Liang et al. 2005).

---

*Corresponding author: Zongqi Liang

Table 1 DNA sequences used in the phylogenetic studies from Genbank

Name	GenBank No.	Name	GenBank No.
P. amoeneroseus	AY624168	*P. inflatus*	AB099943
P. carneus	AY624170	*P. javanicus*	AY624186
P. cateniannulatus	AY624172	*P. lilacinus*	AY624188
P. catenioblíquus	AY624173	*P. marquandii*	AY624193
P. cicadae	AB085887	*P. niphetodes*	AY624192
P. coleopterorum	AY624177	*P. penicillatus*	AY624194
P. farinosus	AY624181	*P. sinensis*	AJ243771
P. fumosoroseus	AY624182	*P. tenuipes*	AY491997
P. ghanensis	AY624185	*P. variotii*	AY247956
P. gunnii	AY489650	*P. viridis*	AY624197

The genus *Paecilomyces* Bainier shares many identical or similar morphological characters with its related genera including *Acremonium* Link, *Gabarnaudia* Samson & W. Gams; *Penicillium* Link, *Mariannaea* G. Arnaud, *Septofusidium* W. Gams and *Verticillium* Nees. It is difficult to distinguish them using only morphological characters, particularly species intermediate between *Penicillium* and *Paecilomyces*. Recently molecular biological techniques, including phylogenetic rDNA sequence analyses, have become efficient ways to identify the intermediate and doubtful species. In this paper, we used both morphology and phylogenetic analysis of rDNA sequences to identify a few *Paecilomyces* species isolated from soil samples and infected insects of Yunnan Province. The results showed that strain GZDX-IFR-A10.1 is a new species named here as *P. parvosporus*. The strain GZDX-IFR-A35.1 was identified as *P. lilacinus*. While the strains GZDX-IFR-466.6 and GZDX-IFR-468.10 were identified as *P. tenuipes* and *P. cateniannulatus* respectively.

Materials and Methods

Tested strains

Four tested strains, GZDX-IFR-A10.1, GZDX-IFR-A35.1, GZDX-IFR-466.1 and GZDX-IFR-468.10 were used in this paper.

Collection and strain isolation

GZDX-IFR-A10.1 and GZDX-IFR-A35.1 were isolated from soil samples of Dali City, Yunnan Province. Two grams of soil were added to a flask containing 20ml sterilized water and glass beads. The soil suspension was shaken for about 10 min and then diluted to concentrations of 10^{-3}-10^{-1}. 1 ml of soil suspension (10^{-3}) was mixed with Martin's medium in a sterilized 9cm diameter Petri dish and incubated at 25C for 5 days. Colonies with *Paecilomyces* conidiogenous structures were transplanted to Martin's slant for purification. GZDX-IFR-466.1 and GZDX-IFR-468.10 were isolated from infected insects from Xishan Park, Yunnan Province. A small amount of spore powder from insect surface was transplanted to Martin's medium by sterilized inoculation needle, and then incubated at about 25C for 3-5 days. The isolates were purified when their conidiogenous structures became the same as those on insects.

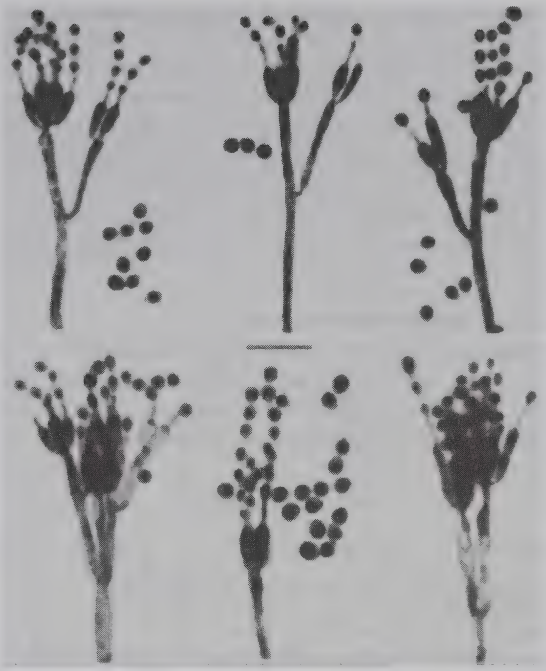


Fig. 1 Conidiogenous structures of *Paecilomyces parvosporus*. Bar = 10 μm.

Strain identification

The strains under study were transplanted on Czapek agar, potato dextrose agar (PDA), and Sabouraud agar according to Brown and Smith (1957) and Samson (1974). After incubation at 25°C for 7 days, the strains were identified based on colony features, conidiogenous structure and biological property. Type strains of GZDX-IFR-A10.1 and its holotypes GZDX-A10.1, the dried plate cultures on Czapek agar, were deposited in the Institute of Fungus Resources, Guizhou University, Guizhou province, China.

Reagent

Taq enzyme and dNTP were bought from Shanghai Sangon, Agarose Gel DNA Purification kit ver 2.0 from TAKARA Company®

DNA extraction

The four strains from Yunnan Province used for the molecular identification were incubated on Czapek agar and potato dextrose agar. Subsequently, the fresh sporulating cultures were used for DNA extraction according to Tigano-Milani et al. (1995), and then DNA were kept at -20°C.

PCR amplification and determination of ITS rDNA sequences

Polymerase chain reaction (PCR) amplification was performed according to the manufacturer's instructions, 50μL reaction system: 10× reaction buffer 5 μL, dNTP 1μL, primer ITS4 1μL, ITS5 1μL, Pfu buffer 0.5 μl, 2μL of template DNA and ddH_2O 39.5 μL. The amplification program: a first step of 94°C for 5 min; then 35 cycles consisted of 94°C for 40 s, 49°C for 40 s, and 72°C for 1 min; and a final step of 72°C for 10 min. To amplify ITS1-5.8S- ITS2 rDNA, the following primers were used: ITS4 (5'-TCCTCCGCTTATTGATATGC- 3') and ITS5 (5'-GGTGAGAG ATT TCTGTGC -3'). PCR products were purified using Agarose Gel DNA Purification kit ver 2.0 according to its procedure (TAKARA Company), and purified DNA samples were sent to Beijing Sunbiotech Co. Ltd. for sequencing. The rDNA sequences of ITS1-5.8s- ITS2 regions of the four strains from Yunnan Province were submitted to GenBank (DQ187951-DQ187954).

Sequence alignment and phylogenetic analysis
Sequences were aligned by Clustal X, and adjusted to maximize homology. Then the phylogenetic tree was constructed using Neighbour-Joining (NJ) and Maximum Parsimony (MP) methods in PAUP 4.0b10. Confidence values for individual branches were determined by bootstrap analysis (1000 replications).

Results

Taxonomy

Paecilomyces parvosporus Y.F. Han & Z.Q. Liang **sp. nov.** **Fig.1**

> *Coloniae in agaro Czapekii ad 20 mm diam, 14 diebus 25°C, primitus albae, tum leteus, olivaceus in margo; reversio citrinae. Hyphae hyalina, levia, 0.6-3.6 μm crassa. Conidiophora ramosa, 11.2-38.1 μm crassa, phialidibus 2 vel 5 terminatis. Phialides 4.8-9.6 × 1.2-1.8 μm, e basi inflata, cylindrical vel clavata in collum tenerum; Conidia continua, hyalina, levia, subglobulosa; vel globulosa, 1.2-2.4 μm. Chlamydospora ellipsoidea, 4.0-10.1 × 2.7-6.7 μm.*
>
> *Typus: GZDX-IFR-A10.1 et cultara GZDX A10.1, isolatus e soli, Dali city, provincia Yunnan, China, VI, 2004, Y.F. Han & Z.Q. Liang. In Guizhou Univ, conservatur.*

Colony on Czapek agar, attaining a diameter of 20mm within 14 days at 25°C, floccose, at first white, then light yellow, green in margin, appearing evident radial furrow, round; reverse yellow. Vegetative hyphae hyaline, smooth-walled, septate, 0.6-3.6 μm in diameter. Conidiophores hyaline, smooth-walled, septate, branched, about 11.2-38.1 μm, bearing whorls containing 2-5 phialides. Phialides 4.8-9.6 × 1.2-1.8 μm, consisting of a cylindrical or claval basal portion, tapering into a distinct neck. Conidia in dry divergent chains, one-celled, smooth, subglobose to globose, 1.2-2.4 μm in diameter; Chlamydospores ellipsoidal, 4.0-10.1 × 2.7-6.7 μm.

> Material examined—GZDX-IFR-A10.1 was isolated by Y.F. Han & Z.Q. Liang from vegetable soils collected in Dali, Yunnan Province, China, June, 2004.

Distribution—Yunnan Province, China.

DNA sequencing

To analyze the ITS rDNA sequence of the tested strains and *Paecilomyces* species from NCBI (Table 1), PAUP was applied to construct the phylogenetic tree by the methods of

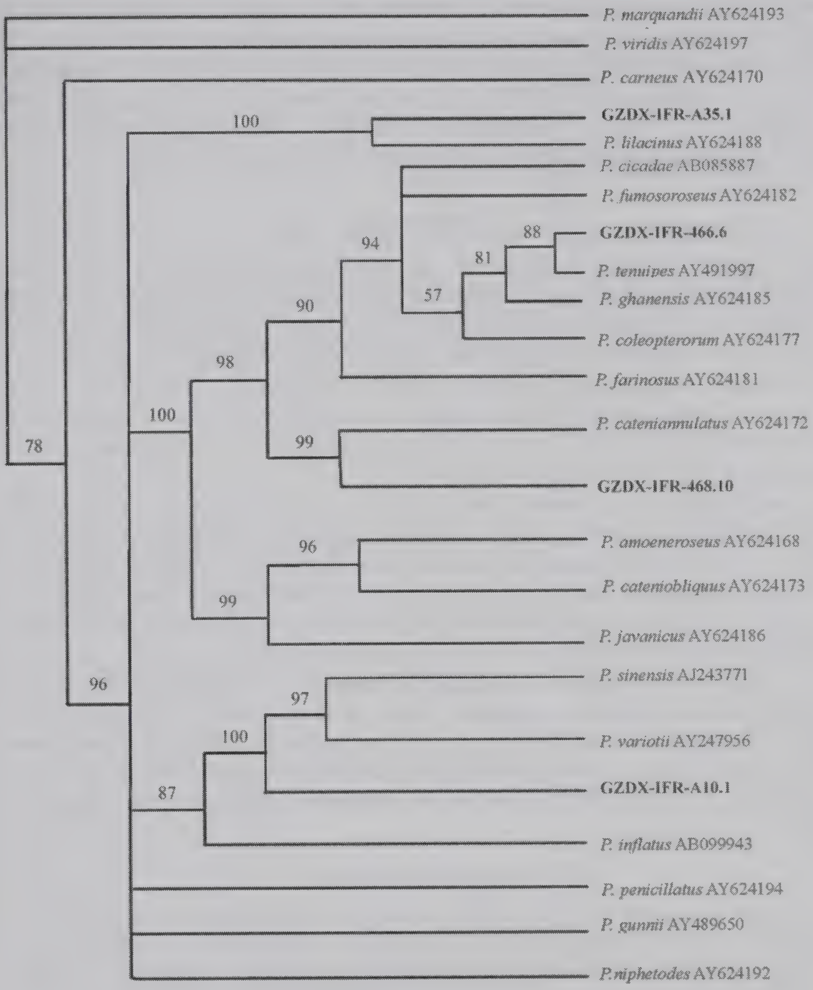

P. marquandii AY624193
P. viridis AY624197
P. carneus AY624170
100
GZDX-IFR-A35.1
P. lilacinus AY624188
P. cicadae AB085887
P. fumosoroseus AY624182
88
GZDX-IFR-466.6
94
81
P. tenuipes AY491997
57
P. ghanensis AY624185
90
P. coleopterorum AY624177
98
P. farinosus AY624181
100
P. cateniannulatus AY624172
78
99
GZDX-IFR-468.10
96
P. amoeneroseus AY624168
P. cateniobliquus AY624173
99
P. javanicus AY624186
P. sinensis AJ243771
96
97
100
P. variotii AY247956
GZDX-IFR-A10.1
87
P. inflatus AB099943
P. penicillatus AY624194
P. gunnii AY489650
P.niphetodes AY624192

Fig.2 Phylogenetic tree based on aligned internal transcribed spacer sequences (ITS1-5.8S-ITS2 rDNA) of selected *Paecilomyces* species, showing the sister group relationship between a few species and the new species. (CI = 0.7284, HI = 0.2716, RI = 0.7577, RC = 0.5520)

NJ and MP. The results from NJ and MP methods are almost identical, so only the tree constructed by MP was shown in the paper. The phylogenetic tree inferred from the ITS sequence data (Fig.2) shows that the two strains GZDX-IFR-466.6 and GZDX-IFR-468.10 are clustered well with *P. tenuipes* AY491997 and *P. cateniannulatus* AY624172 from NCBI in two subclades (88% and 99% bootstrap supports), respectively. GZDX-IFR- A35.1 strain is very similar to *P. lilacinus* in the morphological characters, its ITS sequence is clustered very well with *P. lilacinus* AY624188 in the same subclade (100% bootstrap support), so GZDX-IFR- A35.1 strain is identified as *P. lilacinus*. The strain GZDX-IFR-A10.1 forms a separate branch close to *P. sinensis* AJ243771, *P. variotii* AY247956 and *P. inflatus* AB099943 is therefore identified as a new species.

Discussion

In the genus *Paecilomyces*, based on the morphological characters, the accepted species with cylindrical phialides base and globose conidia are as follows: *P. carneus*. *P. marquandi, P. stipitatus* and *P. vinaceus*. The conidia of *P. carneus* are echinate. The colony of *P. marquandii* is red, but the colony of the new species is yellow to green. The phialides of *P. stipitatus* have septate and thin long stalks, and its colony is white. *P. vinaceus* is characterized by its vinaceous colony reverse. The new species can be distinguished from the above-mentioned species by its greenish colony, much smaller conidia and its very thin, long phialide necks.

The phylogenetic tree showed that the newly named *P. parvosporus* (GZDX- IFR-A10.1), *P. inflatus, P. variotii,* and *P. sinensis* were grouped in the same subclade. But *P. inflatus* has monophialides and fusiform conidia (Samson 1974). *P. sinensis* can produce obvious synnemata on the media and its conidia are fusiform to ellipsoidal, 4.5-7.5 × 1.2-3.8 μm (Q.T. Chen 1984). The 3.2-5 × 2-4 μm conidia and fulvous colony of *P. variotii* (Brown & Smith 1957, Samson 1974) distinguish it from the new species.

In addition, *P. parvosporus* was highly homologous with *Penicillium daleae* when blasted in NCBI Genbank. According to Raper & Thom (1949), *Penicillium daleae* is mainly characterized by coarsely roughened conidia that are ellipsoidal to subglobose, 3.0-3.5 × 2.5-3.0 μm. *P. parvosporus,* however, has smooth conidia that are globlose to subglobose, 1.2-2.4 μm. It can therefore be obviously distinguished from *Penicillium daleae.*

Acknowledgements

This project was supported by the National Natural Science Foundation of China (NSFC No. 30270011) and the Key Laboratory of Microbial Resources, Yunnan University, Yunnan Province, the Ministry of Education P. R. China. We are grateful to Dr. Shengjun Chang and Dr. Jiangxin Wang for their comments on the manuscript. We also appreciate Dr. Jinkui Yang for his help during the experiment, and thank Dr Shaun Pennycook and the Editor-in-Chief for their warm corrections.

References

Brown AHS, Smith G. 1957. The genus *Paecilomyces* Bainier and its perfect stage *Byssochlamys* Westling. Transactions of the British Mycological Society 40: 17-89.

Chen, QT, Xiao RS, Shi ZY. 1984. *Paecilomyces sinensis* sp. nov. and its connection with *Cordyceps sinensi. Acta Mycologica Sinica* 3: 24-28.

Han YF, Liang ZQ, Chu HL. 2005a. Studies on the genus *Paecilomyces* in China II. *Paecilomyces* spp. from Guizhou Mycotaxon 91: 361-364.

Han YF, Chu HL, Liang ZQ. 2005b. Two new species of the genus *Paecilomyces* in China. Mycotaxon 92: 311-316.

Liang ZQ. 1981. Entomogenous fungi from the pests of tea-plants. Acta Phytopathologica Sinica 11(4): 9-16.

Liang ZQ, Chu HL, Han YF. 2005. A thermotolerant *Paecilomyces* sp. nov., which produces laccase and biform of sporogenous structure. Fungal Diversity (In press).

Liu AY, Liang ZQ. 2003. *Paecilomyces amoeneroseus,* one new record in China. Mycosystema 22(Suppl.): 80-81.

Raper KB, Thom C. 1949. The manual of the *Penicillia*. The Williams & Wilkins Co., Baltimore, 296-298.

Samson RA. 1974. *Paecilomyces* and some allied hyphomycetes. Studies in Mycology No.6: 1-119.

Tigano-Milani MS, Samson RA, Martins I, Sobral BWS. 1995. DNA markers for differentiating isolates of *Paecilomyces lilacinus*. Microbiology (Reading, U.K.), 141: 239–245.

MYCOTAXON

Volume 94, pp. 365–370 October–December 2005

Notes on *Otidea* from Xinjiang, China

Wen-Ying Zhuang[1]

zhuangwy@sun.im.ac.cn
Key Laboratory of Systematic Mycology & Lichenology, Institute of Microbiology
Chinese Academy of Sciences, Beijing 100080, China

Abstract — Newly collected specimens of *Otidea* from Xinjiang, China were examined. Five taxa of the genus are identified. Among them, *Otidea crassa* is described as a new species and *O. onotica* var. *brevispora* is treated a new variety.

Key words — taxonomy, new taxa

Introduction

Species of the genus *Otidea* (Pers.) Bonord. is widely distributed in north temperate areas. They are characterized by apothecia of medium to large size, mostly terrestrial, ear-shaped, split down to base or discoid, with some shade of whitish, yellow, brown or ochre; asci operculate, not blued by iodine; ascospores elliptical to elliptical-fusiform, biguttulate; paraphyses slender, strongly curved at apex, capitate or with notches at apical portion. According to the CABI database,[2] 71 published species names are listed under *Otidea* and apparently 17 of them should be excluded from the genus. But many of the rest are either synonyms or doubtful members of the genus. About 15 species are commonly accepted in the world (Kirk et al. 2001). Regional studies have been carried out in China, India, Japan, Lithuania, United Kingdom, the Nordic countries, North America, etc. (Kanouse 1949, Nannfeldt 1966, Otani 1969, Thind & Waraitch 1974, Harmaja 1976, Dennis 1978, Breitenbach & Kränzlin 1984, Cao et al. 1990, Korf & Zhuang 1991, Dissing 2000, Kutorga 2000). Species diversity of the genus in each region seems not very high. In China, fifteen taxa of *Otidea* were previously reported by Cao et al. (1990) from Gansu, Heilongjiang, Jilin, Shaanxi, Shanxi, Sichuan, Yunnan, Xinjiang, and Xizang. *Otidea alutacea* var. *alutacea* [2 collections] and *O. propinquata* (P. Karst.) Harmaja [a single collection] in *Picea* forests were the only records known from Xinjiang, an area in the northwest occupying more than 1/7 of the China mainland territory. In our recent field trip to Xinjiang, 20 specimens of the genus were collected from Burqin, Jimsar, and Yining. Five taxa are recognized among them. *Otidea leporina* is the most common. *Otidea crassa* is described as a new species and *Otidea onotica* var. *brevispora* is proposed as a new variety.

Supported by the National Natural Science Foundation of China (nos. 30230020, 30499340).

[2] http://www.indexfungorum.org/Names/Names.asp

Taxonomy

Otidea alutacea (Pers.) Massee, British Fungus Flora 4: 446, 1895.

> Specimens examined: CHINA. Xinjiang, Urmuqi, on the ground, 12 VIII 1985, L. Fan 10, HMAS 88262; Xinjiang, Burqin, Hemuxiang, alt. 1100 m, 5 VIII 2003, on the ground, W. Y. Zhuang & Y. Nong 4719, 4722, 4685, HMAS 83559, 83560, 83562; *ibid.*, 6 VIII 2003, on the ground, W. Y. Zhuang & Y. Nong 4744, 4739, HMAS 83561, 83563.

Notes: The fungus is characterized by the truncate to cupulate apothecia with one side split to the base and up to 5–7.5 cm wide; hymenium surface beige, light cinnamon, cinnamon brown to brown when fresh; receptacle surface usually lighter than hymenium surface, beige, light cinnamon brown, brownish yellow to brown; ascospores ellipsoid with blunt ends, 13.5–15.8 × 6–8.5μm; paraphysis apices curved to hooked. The five known collections were all found in Burqin in northeastern Xinjiang. It was reported previously from Heilongjiang, Jilin, Xinjiang (Urmuqi), Shanxi, and Xizang of the country (Cao et al. 1990).

Otidea cochleata (L.) Fuckel, Jahrb. Nassauischen Vereins Naturk. 23-24: 329, 1870.

> Specimens examined: CHINA. Xinjiang, Jimsar, alt. 1700 m, 1 VIII 2003, on duff under *Picea* sp., W. Y. Zhuang & Y. Nong 4622, 4645, 4651, HMAS 83564, 83575, 83576.

Notes: This species is characterized by the medium-sized, cupulate to truncate apothecia with light brown, brown to dark brown hymenium surface and light brown, yellowish brown to brown receptacle surface when fresh; hymenium 240–270 μm thick, asci 12.5–15 μm wide; and ellipsoid to broad-ellipsoid ascospores 15–18 × 8.6–11.7 μm. The club-shaped cells of the outer excipulum arise from small angular cells which arise by a gradual transition from the textura intricata of the medullary excipulum.

Otidea cochleata was first reported from Milin, Xizang in southwestern China based on one collection (Cao et al. 1990). *Otidea umbrina* (Pers.) Bres. is a later synonym according to the CABI database.

As noted by Breitenbach & Kränzlin (1984), *Otidea cochleata* is similar to *O. alutacea* in apothecial shape and is distinguished from the latter in somewhat smaller and darker fruitbodies, as well as the larger ascospores.

O. cochleata might also be confused with *O. propinquata* (P. Karst.) Harmaja occurring in a similar niche. The latter possesses larger ascospores usually over 18 μm in length (Velenovský 1934, Kanouse 1949, Harmaja 1976). The single specimen from Xinjiang previously recorded as *O. propinquata* (Cao et al. 1990) has not been located. Another collection identified at the same time as *O. propinquata* from Shanxi turns out to be *O. cochleata*.

Otidea crassa W.Y. Zhuang, **sp. nov.** **Figs. 1–3**

> *Ab* Otidea leporina *surperficie hymenii flavo-brunneis vel brunneis, surperficie receptaculi dilute flavo-brunneis, ascis 178–192 × 9.5–12.5 μm, ascosporis late ellipsoideus, brevibus, 10.5–12.5 × 7.4–9 μm differt.*
>
> Etymology: The specific epithet refers to the broadly ellipsoid ascospores produced by the fungus.

Apothecia ear-shaped, short-stipitate, 1.2–2 cm wide and up to ca 3 cm high, hymenium surface yellowish brown to brown when fresh and light brown to warm brown when dry, receptacle surface paler than hymenium when fresh, pale yellowish brown to pale brown when fresh and becoming orange-brown to warm brown when dry, stipe base whitish; ectal excipulum of textura angularis, 40–90 μm thick, cells more or less isodiametric, 8–30 μm diam.; medullary excipulum of textura intricata, 240–330 μm thick, hyphae hyaline, 3–12.5 μm wide; subhymenium 30–50 μm thick; hymenium 165–215 μm thick; asci 8-spored, subcylindrical, J– in Melzer's reagent, ca 178–192 × 9.5–12.5 μm wide; ascospores broadly ellipsoid with rounded or blunt ends, biguttulate, smooth, uniseriate, 10.5–12.5 × 7.4–9 μm; paraphyses filiform and curved at apex, 2–2.5 μm wide at apex and ca 1.5–2 μm below.

> Holotype: CHINA. Xinjiang, Jimsar, alt. 1700 m, 1 VIII 2003, on mossy rotten wood, W. Y. Zhuang & Y. Nong 4647, HMAS 83571. Paratype: CHINA. Xinjiang, Jimsar, alt. 1700 m, 1 VIII 2003, on mossy rotten wood, W. Y. Zhuang & Y. Nong 4631, HMAS 83570.

Notes: The new species is similar to *Otidea leporina* (Kanouse 1949) except for the thinner flank, yellowish brown hymenium surface, and broadly ellipsoid ascospores. When the two collections of *O. crassa* were compared with eight of *O. leporina* from Xinjiang, distinctions have been found in spore size and shape [10.5–12.5 × 7.4–9 μm vs. 11.5–13.5 × 6.4–8 μm in the Chinese collections], width of medullary excipular hyphae (3–12.5 μm vs. 2.5–5 μm wide), size of ectal excipular cells (8–30 μm in diameter vs. 6–15 μm wide), and its occurrence on rotten wood instead of soil and duff.

In addition to the morphological characteristics, our 28S nrDNA partial sequence data (unpublished) support also its separation from *O. leporina* and show close relationship to *Otidea cantharella* (Fr.) Quél., a species with bright yellow pigment in fruitbodies and composed of only one kind of tissue in excipulum, viz. outer layer of *textura prismatica* changing gradually to inner layer of *textura intricata*, which was treated in a separate genus as *Flavoscypha cantharella* (Fr.) Harmaja (Harmaja 1974).

The apothecial shape and spore size of the new species are also similar to those of *O. felina* (Pers.) Bres. but the fruitbodies are smaller (1.2–2 cm wide and up to ca 3 cm high vs. 2–3 cm wide and 3–4.5 cm high), apothecial color is yellowish brown to brown when fresh instead of light grayish yellow to light flesh colored, ascospores are wider, shorter [10.5–12.5 × 7.4–9 μm vs. 11–13 × 6–8 μm according to Saccardo 1913)] and broadly ellipsoid with blunt ends instead of ellipsoid with slightly pointed ends (Boudier 1905-1910).

Otidea leporina (Batsch) Fuckel, Jahrb. Nassauischen Vereins Naturk. 23–24: 329, 1870 var. ***leporina***

> Specimens examined: CHINA. Xinjiang, Jimsar, alt. 1700 m, 1 VIII 2003, on the ground, W. Y. Zhuang & Y. Nong 4632, 4633, 4634, 4657, 4635, 4636, 4637, HMAS 83565, 83566, 83567, 83568, 83577, 83578, 83579.

Notes: The species seems very common in Xinjiang, as well as in other areas of China, such as Heilongjiang, Hunan, Jilin, Shanxi, Sichuan, Xizang, and Yunnan as reported by Cao et al. (1990) and Zang (1996). It is characterized by the ear-shaped apothecia 2–4 cm wide, with hymenium surface cinnamon, cinnamon brown, warm brown,

brown to grayish brown when fresh and receptacle surface concolorous, dark brown or grayish yellow; ectal excipulum of textura angularis, cells 6–15 µm wide, outermost cells sometimes short club-shaped; medullary excipulum of textura intricata, hyphae stable in width, ca 2.5–5 µm wide; hymenium 165–178 µm thick; asci 8–10 µm wide; and ascospores ellipsoid, 11.5–13.5 × 6.4–8 µm; paraphyses curved, 1.5–2.5(–3) µm wide at apical portion.

HMAS 83569 (Xinjiang, Yining, Guozigou, alt. 1800 m, 11 VIII 2003, W. Y. Zhuang, Y. Nong & S. Y. Guo 4845) has smaller, ear-shaped, brown to grayish brown fruitbodies up to 1.5 × 0.8 cm; elongate-ellipsoid, longer ascospores 12.5–16 × 6–8 µm; and paraphyses straight, bent or curved at apex and 2.5–3.2 µm at the widest, and is here tentatively treated as *Otidea* cf. *leporina*.

Kanouse (1949) accepted Saccardo's (1889) separation of *Otidea leporina* var. *minor* (Rehm) Sacc. from the original variety. The Chinese collections appear to have identical morphology to *O. leporina* var. *leporina*.

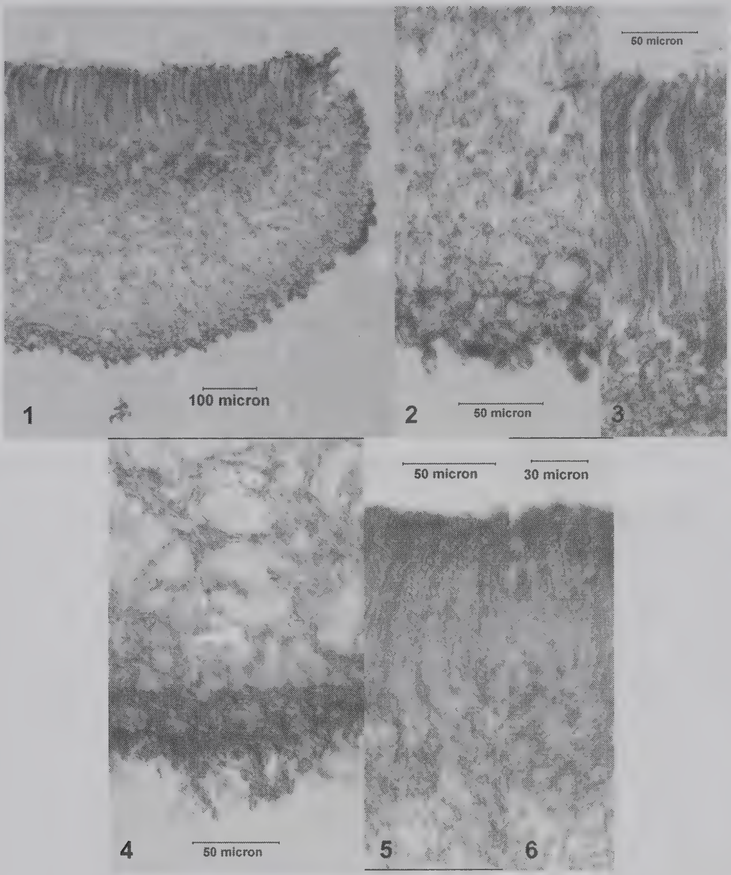
Otidea onotica var. ***brevispora*** W.Y. Zhuang, **var. nov.** **Figs. 4–6**

> *Ab* Otidea onotica *var. onotica ascosporis brevibus, ellipsoideus vel late ellipsoideus, 8.5–10.5(–11) × 5.2–7 µm differt.*
>
> Etymology: The specific epithet refers to the short ascospores produced by the fungus in comparison with those in *Otidea onotica* var. *onotica*.

Apothecia spoon- to ear-shaped, short-stipitate, 1–5 cm wide and 2.5–8 cm high, hymenium surface yellow to dull yellow when fresh, receptacle surface yellow or paler than hymenium when fresh, stipe base whitish; ectal excipulum of textura angularis, 50–80 µm thick, cells 13–20 × 5–12 µm and 9–26 µm in diameter if isodiametric, with the outer most cells somewhat club-shaped; medullary excipulum of textura intricata, 100–660 µm thick, hyphae hyaline, 2–8 µm wide; subhymenium 40–50(–70) µm thick; hymenium 140–180 µm thick; asci 8-spored, subcylindrical, J– in Melzer's reagent, (7–)8–9.5(–10.5) µm wide; ascospores ellipsoid to broadly ellipsoid with rounded or blunt ends, biguttulate, smooth, uniseriate, 8.5–10.5(–11) × 5.2–7 µm; paraphyses filiform and curved at apex, 2–2.8 µm wide at apex and ca 1.5 µm below.

> Holotype: CHINA. Yunnan. Baoshan, alt. 1900 m, 24 VII 2003, Z. L. Yang 3854, HKAS 43003, isotype HMAS 83551. Paratypes: CHINA. Heilongjiang, Yichun, 6 IX 1989, on the ground under mixed woods, J. Z. Cao 821, HMAS 83572; Xinjiang, VIII 1994, on duff, J. Y. Wang 132, HMAS 69951 (filed as *Otidea cantharella*); Xinjiang, Jimsar, alt. 1700 m, 1 VIII 2003, on the ground, W. Y. Zhuang & Y. Nong 4655, HMAS 83573; *ibid.*, 2VIII 2003, on cone of *Picea* sp., W. Y. Zhuang & Y. Nong 4670, HMAS 83574.

Notes: The fungus is characterized by its ear-shaped, yellow apothecia and shorter ascospores 8.5–10.5(–11) × 5.2–7 µm than those of *Otidea onotica* var. *onotica* [(10–)12–13 × 5–6 (Dennis 1978), 12–14 × 6–7(–8) µm (Kanouse 1949)]. Our 28S rDNA partial sequence data (unpublished) also support its separation from *O. onotica* var. *onotica*.



Figs. 1-6. *Otidea* spp. 1-3. *Otidea crassa* (HMAS 83571): 1. Longitudinal section of apothecium. 2. Excipular structure. 3. Portion of hymenium showing asci and ascospores. 4-6. *Otidea onotica* var. *brevispora* (HMAS 83551): 4. Excipular structure. 5, 6. Portion of hymenium showing asci and ascospores.

Acknowledgements

The author would like to thank Prof. R. P. Korf and Prof. D. H. Pfister for serving as pre-submission reviewers, Dr. Z. L. Yang for providing his own collection from Yunnan, Mr. Y. Nong for collecting jointly the specimens used in this work, Mr. C. Y. Liu for providing information about *Otidea* DNA sequences, and Ms. X. Song for making the sections.

Literature cited

Boudier E. 1905-1910. Icones Mycologicae. Tome II. Paris.

Breitenbach J, Kränzlin F. 1984. Fungi of Switzerland. Vol. 1 Ascomycetes. Luzern. [English transl.]

Cao JZ, Fan L, Liu B. 1990. Some species of *Otidea* from China. Mycologia 82: 734-741.

Dennis RWG. 1978. British Ascomycetes. ed. 2. Cramer. Vaduz.

Dissing, H. 2000. Pezizales. In Hansen L, Knudsen H. (eds.). Nordic Macromycetes. Vol. 1. Nordsvamp, Copenhagen.

Harmaja H. 1974. *Flavoscypha*, a new genus of the Pezizales for *Otidea cantharella* and *O. phlebophora*. Karstenia 14: 105-108.

Harmaja H. 1976. New species and combinations in the genera *Gyromitra*, *Helvella* and *Otidea*. Karstenia 15: 29-32.

Kanouse BB. 1949. Studies in the genus *Otidea*. Mycologia 41: 660-677.

Kirk PM, Cannon PF, David JC, Stalpers JA (eds.). 2001. Ainsworth & Bisby's Dictionary of the Fungi. Edition 9. CAB International, Surrey UK.

Korf RP, Zhuang WY. 1991. A preliminary discomycete flora of Macaronesia: Part 15, *Terfeziaceae*, and *Otideaceae, Otideoideae*. Mycotaxon 40: 413-433.

Kutorga E. 2000. Mycota Lithuaniae. *Pezizales*. Bot. Inst., Vilniaus Univ. Vilnius. (in Lithuanian)

Nannfeldt JA. 1966. On *Otidea caligata*, *O. indivisa* and *O. platyspora* (Discomycetes Operculatae). Ann. Bot. Fenn. 3: 309-318.

Otani Y. 1969. Some species of the genus *Otidea* collected in Japan. Trans. Mycol. Soc. Japan 9:101-108.

Saccardo PA. 1889. Sylloge Fungorum. Vol. 8. Saccardo. Padova.

Saccardo PA. 1913. Sylloge Fungorum. Vol. 22. Saccardo. Padova.

Teng SC. 1963. Fungi of China. Science Press. Beijing. (in Chinese)

Thind KS, Waraitch KS. 1974. The *Pezizales* of India – XIII. Proc. Nat. Acad. Sci. India 44(B): 13-21.

Velenovský J. 1934. Monographia Discomycetum Bohemiae. Pragae.

Zang M (ed.). 1996. Fungi of the Hengduan Mountains. Science Press. Beijing. (in Chinese)

MYCOTAXON

Volume 94, pp. 371–391 October–December 2005

Book reviews and notices

Compiled by

David L. Hawksworth[1]

MycoNova, The Yellow House, Calle Aguila 12,
Colonia La Maliciosa, Mataelpino
ES-28492 Madrid, Spain.

General

Mycelium Running: How Mushrooms can help Save the World. By Paul Stamets. 2005. Ten Speed Press, Box 7123, Berkeley, CA 94707, USA. Pp. x + 339, figs 360. ISBN 1 58008 579 2. Price: US $ 35.

This book by Paul Stamets, an entrepreneur and fellow fungus chauvinist, is not a systematic text, but is mentioned here as it is something that helps promote the importance of mycology in ecological processes and human settings. He draws parallels of mycelial networks with the internet, and stresses the potential of fungi in mycofiltration, mycoforestry, mycoremediation, and as mycopesticides. All this is written with a racy style and well-illustrated by colour photographs and drawings. The next two thirds of the book is devoted to the cultivation of mushrooms, and their nutritional and medicinal properties -- including many references to original sources. In summary, a great book to direct someone to who is unsure why on earth anyone should take an interest in mushrooms. A limited edition of 200 individually numbered, signed, boxed and hardbound versions is also available at US $ 195.

Biodiversity of Fungi: Their Role in Human Life. Edited by Sunil K. Deshmukh & Mahendra K. Rai. 2005. Science Publishers, Enfield, P. O. Box 699, NH 03784, USA. Pp. 461. ISBN 1 57808 368 0. Price: £ 48.40.

This volume comprises a hotchpotch of 18 chapters, some of which do emphasise biodiversity, others do concern their impact on the human environment, and some manage both at the same time. How some chapters, e.g. 'Fungal protoplast technology', fall under the umbrella title is not immediately obvious. The book is certainly a 'curate's egg', good in parts. Elizabeth Arnold's 'Diversity and ecology of fungal endophytes in tropical forests' was a highlight.

---

[1] Books for consideration for coverage in this column should be mailed to the Book Review Editor (address above) in the first instance. Fax (+34) 91 857 3640; e-mail: myconova@terra.es.

Gunde-Cimarmans *et al.* 'Halotolerant and halophilic fungi', Deshmukh & Kushwaha 'Keratinophilic fungi on birds and their ability to decompose keratin', Wasser & Didukh's 'Mushroom polysaccharides in human health care', and Maldonades & Ibarra's 'Organic dyes from fungi and lichens' truly embraced the diversity theme. There are also useful chapters on the potential of ligninolytic basidiomycetes to degrade organopollutants, and on metal bioremediation (but why the latter needed three chapters I do not know).

There seems to be an increasing number of books that lump a set of loosely related chapters together under a catchall title, with the assumption that this provides a valuable contribution to the literature. Sadly, more often than not it does not work. Multi-author books need to provide more than the sum of the individual parts. This book is reasonably priced, but I will not be recommending it for purchase by the library.

LYNNE BODDY
Cardiff School of Life Sciences, Main Building,
Park Place, Cardiff CF10 3TL, UK

Fungi in Forest Ecosystems: Systematics, Diversity, and Ecology. Edited by Cathy L. Cripps. 2004. The New York Botanical Garden Press, 200th Street and Kazimiroff Boulevard, Bronx, New York, NY 10458-5126, USA (e-mail: nybgpress@nybg.org). Pp. xvi + 363. [Memoirs of the New York Botanical Garden Vol. 89.] ISBN 0 89327 459 3. Price: US $ 68.

This volume has been prepared as a tribute to Orson K. Miller Jr, 75 years young in 2005. Following a detailed, well-illustrated[2], and at times somewhat light-hearted life history, there are lists of theses prepared under his direction, new scientific names he has introduced, and a bibliography of his publications. The original papers contributed mainly by close friends and colleagues are placed in three groups: Systematics, Diversity, and Ecology. Those on systematics include ones on *Amanita* subgen. *Lepidella, Crepidotus, Entoloma,* and *Mycena,* between them with descriptions of 17 new species, mostly macromycetes. The diversity chapters cover different groups of fungi in areas from Papua New Guinea, through the European alps and Israel, to Oregon and the southern Appalachians. The contributions in the ecology group mainly concern ectomycorrhizas or wood-decay fungi. There are some fascinating items amongst the papers, from the relation of *Phellinus pini* to red-cockaded woodpecker cavity trees to macrolichen distribution patterns in the Montana and Yellowstone and Glacier National Parks, and an intriguing winter-fruiting helotialean anamorph (*Cadophora hiberna* sp. nov.). This is a work that all mycological libraries with a macromycete focus should endeavour to acquire, not least for its compilations of information on Orson's outstanding contributions.

Fungi: Experimental Methods in Biology. By Ramesh Maheshwari. 23 June 2005. CRC Press, Taylor & Francis Group, 6000 Broken Sound Parkway NW, Suite 300, Boca Raton, FL 33487-2742, USA. Pp. xii + 240. [Mycology Series Vol. 24.] ISBN 1 57444 468 9. Price: US $ 149.95, £ 85.

---

[2] I especially liked the photograph of Orson at the microscope under the caption "Girl Scout Leader".

While the subtitle might lead to the expectation that this was a hands-on book on methodologies, it is actually a textbook that arose from the teaching of graduate students in biochemistry in Bangalore. Those students would have clearly been especially favoured to have had such an exposure to mycology, but sadly much seems to be tailored to such students' perceived needs rather than providing a balanced introduction to the subject. The book is divided into six parts and an appendix. These address: The unique features of fungi (2 chapters), Integration of fungi with other organisms (2), Model fungi in research (4), Gene manipulation in fungi (1), Adaptations (3), and Populations (2). It would not be pertinent to consider these sections in depth in *Mycotaxon,* but I have to say that I was frankly aghast at the appendix on Naming, defining and broadly classifying fungi. Most of the appendix consists of crude illustrations evidently scanned from poor quality Xerox copies of earlier works, incorrectly uses subphylum terminations (i.e. '-*mycotina*) for the main groups labelled as at the rank of phylum (names in which have the termination '-*mycota*). Then, to compound matters, the author recognizes 'phylum *Deuteromycotina* (*Fungi Anamorphici*)! Just how out of touch can an author be with current thinking? The 2001 edition of *Ainsworth & Bisby's Dictionary of the Fungi* is cited at the end of the appendix, but clearly has not been taken note of.

Further, in the main body of the work, to find no detailed consideration of molecular phylogenetic approaches is almost unbelievable for a work with a 2005 date – there is only one tree from a paper of the author's from 2002 on Indian *Neurospora* strains accompanied by a mere 15 lines of discussion. Even with all the exciting current experimental work, mycorrhizas get a mere seven pages, and the part on interactions with other organisms does not have anything on insect-fungus relationships or medical aspects. There is even no treatment of endophytic fungi which I would have expected biochemistry students to want. In addition, there are numerous mistakes and out of date information in other sections, and I fail to see why the same photographs have been included twice in some cases, once in black and white and once in colour (e.g. Fig. 3.5, also not named in the legend, but probably *Caloplaca thallincola* growing on *Verrucaria maura*).

This is a stain in an otherwise really splendid series, and neither the text nor the figure quality can have been critically reviewed prior to publication. This is sad as the series would have benefited from counting a rounded text amongst its volumes, especially as there is still a shortage of authoritative introductions to the subject. Fortunately, the price of the book should prevent most students' having access to a copy.

Revue du Cercle de Mycologie de Bruxelles. Numéro 1. September 2001. Cercle de Mycologie de Bruxelles, c/o Yolande Mertens, Tomberg 116 bte 12, B-1200 Bruxelles, Belgium. Pp. 52. ISSN not indicated. Annual membership: 12.50 €.

This new journal is mentioned here as it is full of systematic papers, rather than just excursion reports and notes on interesting finds. And it deals no just with macromycetes. The first number has five original papers which concern, *Amanita vittadinii, Caloscypha fulgens, Podospora conica, Puccinia albescens,* and *Sarcoscypha jurana.* The second, issued in 2002, includes systematic contributions on *Ascobolus degluptus, Cordyceps* (with a key to 14 species and distribution maps), *Discinella lividopurpurea, Gymnosporangium sabinae,* and a key to long-stalked *Inocybe* species based on spore characters. Where there are field meeting reports, these include notes on the features of interesting species. Colour photographs illustrate most articles. The journal is edited to a high professional standard, should be in all major mycological libraries, and merits coverage in abstracts databases.

Encyclopedia of Fungi of Britain and Europe. By Michael Jordan. 2004. Revised edn. Frances Lincoln, 4 Toriano Avenue, London NW5 2RZ, UK. Pp. 384, numerous col. photographs. ISBN 0 7112 2378 5 (hardback), 0 7112 2379 3 (paperback). Price: £ 27.50 (hardback), £ 15 (paperback).

When this work first appeared in 1995 it was warmly welcomed by the amateur mycological community because of its excellent colour photographs and concise descriptions. As in all identification guides covering a wide range or fungi, there were invariably some errors in the applied names, and this led to some most unfortunate and disproportionate published criticisms and dissociations (e.g. Emmett 1996; Henrici 1996). It is always hard for someone without the resources of a major institution behind them to produce such a major work, and such texts have to be seen in that context. For this revised edition, the author has endeavoured to address the criticisms and updated about 60 photographs and replaced or relocated some eight that were wrongly named.

The fungi are arranged by major group, and while "*Aphyllophorales*" and "*Gasteromycetes*" appear as categories for practical reasons, I was very pleased to see these names in inverted commas and the correct disposition of genera provided (e.g. *Lycoperdon* in *Agaricales*).

While the focus of the book is the photographs and descriptions, there are also helpful introductory chapters with notes on nomenclature, collecting, photography, chemical tests and conservation, as well as a colour chart and glossary. There is also a key and bibliography, although the latter sadly has not been updated and no post-1993 works included: further, *Ainsworth & Bisby's Dictionary of the Fungi* is given as "1986", ignoring the 1995 and 2001 editions.

Interestingly for a book aimed at amateur mycologists and mycophagists, English common names are generally omitted, as "for the vast bulk of fungi the Latin name is the only acceptable option" – I concur.

With so many fine photographs and at such a reasonable price in paperback, this revised edition will do much to promote serious mycological studies amongst the amateur community, those who are the key to the much deeper understanding of macromycete distribution and ecology that is so badly needed.

This is a fine achievement on which the author and publisher are to be congratulated, and those that might wish to pinprick from advantageous standpoints need to stand back and see the work in context. I did not consider it appropriate so to do, and look forward to a succession of future editions.

Emmett, E. E. (1996) A disclaimer. *Mycologist* **10**: 95.

Henrici, A. (1996) The Encyclopedia of Fungi. *Mycologist* **10**: 183.

Die Pilzflora des Ulmer Raumes. By Manfred Enderle. 2004. Manfred Enderle, Am Wasser 22, D-89340 Leipheim-Riedheim, Germany (e-mail: manfred.ender. Pp. 521, illustrated. ISBN 3 88294 336 X. Price: 24.50 €.

This beautifully presented and personal work aims to document the range of fungi so far found in the Ulm area of Baden-Württemberg in Bavaria, Germany. After a short but clear and well-illustrated introduction to what fungi are and characters used in macromycete identification, a detailed history of fungus exploration in the area follows. The first records go back to 1728, but the account goes through to document the activities of the local mycological society, the Arbeitsgemeinschaft Mykologie Ulm (AMU) founded in 1976, including data in publications and the personalities involved. There are also lists of localities with MTB grid square references, and sections on geology, soils, climate, and vegetation types – the latter with lists of species frequent in or characteristic of them within.

The core of the book is the species lists, which cover slime moulds, and straminipilous fungi as well as asco- and basidiomycetes. Sadly, a category *Fungi imperfecti* is retained, which for some inexplicable reason includes zygomycetes, and lichen-forming fungi are not covered at all. The total number of species treated is given as 2681 species, plus 72 that are unclear or partly described taxa. Most have been collected over the last 40 years, and detailed information is provided on localities, hosts, and for rarer species specific collections with date, collector, place where voucher material is held, or literature reference. Author citations are provided, but unfortunately do not follow those recommended by and used in the *Index fungorum* database – and there are inconsistencies in how the same author is abbreviated.

Fine colour photographs and line drawings are scattered throughout the body of the text, and in some cases full morphological and microscopical descriptions are provided, especially for species that either are in the Red List or described from the region. Critical collecting over an extended period has led to the discovery of a considerable number of novel taxa in the area, and a list of the 43 new scientific names introduced by the author over the years is provided (pp. 64-65). Further, several new taxa are introduced in the book: two new species of *Coprinus s. lat.*, a new variety of *Hebeloma vaccinium,* and a new combination for a form of *Tricholoma vaccinum.*

There are two separate sections of colour plates with integral descriptions at the end of the systematic treatment: one for the 50 most edible species with the outside margins of the pages with a green strip, and one for harmful ones with a red strip. The reference list and full index is preceded by a most delightful 20-pages of half-tone photographs of mycologists on excursions and in meeting rooms or laboratories. Not all are dated, but those that are cover the period 1980-2003 and include many well-known mycologists, several of whom are now dead. Additional snippets are ten rules for collectors, frequently consulted works, and a glossary.

The acknowledgements list is extremely extensive and includes not only different mycologists who have helped, but further several major companies in the region who made financial contributions to the cost of publishing this book – resulting in the most reasonable price for such a well-produced and lavishly illustrated local treatment.

The author should feel well-satisfied with what is very much a testimony and personal record of his four decades of collecting fungi in the Ulm region. It is a "must have" for those working in the region!

Fungi of Northwestern China. Edited by Wen-Ying Zhuang. 2005. Mycotaxon, P.O. Box 264, Ithaca, NY 1485-0264, USA. [Order from: Professor Wen-Ying Zhuang, P. O. Box 2714, Beijing 100080, Peoples' Republic of China (e-mail: zhuangwy@sun.im.as.cn).] Pp. vi + 430, figs 5. ISBN 0 930845 14 5. Price: US $ 40.

The region covered in this compilation embraces Gansu, Ningxia, Qinghair, Shaanxi, and Xinjiang provinces, more than a quarter of the People's Republic of China and including desert areas such as the Gobi desert, grasslands, mountains, and plateaux. In all, 3887 species distributed through 759 genera are treated, including straminipiles, lichen-fungi, and slime moulds. The data are presented as a series of 14 complementary chapters prepared by different specialists, but the editor clearly has insisted on a standard style, so that each entry includes a note on the host or substrate, provinces where the species has been found, and information as to the source (i.e. to a reference collection or publication). Especially helpful are the maps showing collecting sites in each province.

All chapters start with an overview of the study of a particular group in China, and there are comprehensive indices at the end by both host and fungal taxon. This is clearly an important regional synthesis, and will also be an aid to identification through the host index, but very many more species are to be expected. That the text is in English and with extensive references to the literature in Chinese is a real bonus for non-Chinese speakers, making otherwise inaccessible data available. My one frustration is the continual citing of authors of hosts names in the text that have surely never been verified by the authors. Further, abbreviations used for the authors of fungal names are inconsistent, sometimes even within the same chapter.

The work is a complement to *Higher Fungi of Tropical China* (Zhuang 2001; reviewed in *Mycotaxon* 87: 493-494, 2003), and it is to be hoped that in due course it will be followed by parallel treatments of the remaining regions of China.

Zhuang, W.-Y. (2001) *Higher Fungi of Tropical China*. New York: Mycotaxon.

Fungi of the Antarctic: Evolution under Extreme Conditions. Edited by G. Sybren de Hoog. 2005. Centraalbureau voor Scvhimmelcultures, P. O. Box 85167, 3508 AD Utrecht, The Netherlands (e-mail: info@cbs.knaw.nl). [Studies in Mycology No. 51.] Pp. vii + 79. ISBN 90 70351 55 2. Price: 40€.

The two systematic monographs that comprise volume 51 of *Studies in Mycology* provide an outstanding introduction to the world of non-lichenized fungal life under extreme Antarctic conditions. Both groups of fungi are poorly understood, but the results reported in these articles indicate they constitute a substantial part of the microbial diversity of these habitats. I much appreciate the inclusion of both reviews in the same volume since despite the very different strategies of the fungi involved, they share the ability to withstand the harsh conditions of the Antarctic desert, providing new insight into the limits of fungal life. At the end of each article, the authors provide hypotheses for the evolution of the fungal groups treated.

Fungi at the edge of life: cryptoendolithic black fungi from Antarctic desert (L. Selbmann, G. S. de Hoog, A. Mazzaglia, E. I. Friedmann and S. Onofri): The authors present an extensive treatment of Antarctic cryptoendolithic communities. These fungi of diverse phylogeny colonize rock cavities and thus avoid the inhospitable conditions of the rock surface. The article starts with an introduction in which the ecological niche of these fungi is described, along with some of their morphological and physiological characteristics. This is followed by a detailed description of the sampling sites and the methods employed in the study. The results include light microscopy and scanning electron microscopy descriptions of twenty-six strains of black, mostly meristematic, fungi from cryptoendolithic lichen-dominated communities of Antarctica, and the sequencing of their ITS rDNA region. The phylogenetic positions of these different strains and their survival strategies are discussed, and the new genus *Cryomyces* is introduced for two newly described species. A further new species of *Friedmanniomyces* is also named here.

Evolution, taxonomy and ecology of the genus Thelebolus in Antarctica (G. S. de Hoog, E. Göttlich, G. Platas, O. Genilloud, G. Leotta and J. van Brummelen): These authors report a high frequency of *Thelebolus* species in Antarctic "biomats" from several types of lake, and offer an explanation for this based on bird vectors. A short introduction to the genus is followed by a description of the sampling sites and methods used to sample, isolate, and analyze strains, and herbarium collections. The results include an extensive molecular analysis aimed at resolving the phylogeny of this adapted group of cup fungi using different methods, including microsatellite fingerprinting and the sequencing of several genes. Phylogenetic relationships, taxonomic positions and the evolution of this genus are discussed in the light of SSU and ITS rDNA and ß-tubulin sequence data. The article ends with a taxonomic section, which includes a key for the morphological identification of the four species described, two of which are described as new to science and appear to be endemic to Antarctica.

Asunción de los Ríos
Instituto de Recursos Naturales
Centro de Ciencias Medioambientales
Serrano 115dpdo, , Madrid-28006, Spain

Basidiomycetes

Les Réactifs Mycologiques. Vol. 2. **Les Réactifs Microchimiques.** By Jacques Charbonnel. 2004. Edition Jean-Claude David-Rogeat, Rue Général Dufour 131, CH-2502 Bienne, Switzerland (e-mail: info@mycologie.ch). Pp. 289. ISBN 2 9700411 0 2. Price: 50 €.

This is the second part of *Aide pratiques à l'étude microscopique des Champignons* and follows a long French tradition for the use of chemicals in macromycetology, from Bataille to Josserand and Henry *et al.* Indeed 36 mycologists experienced in this field are thanked in the opening pages.

There are seven chapters, the first setting out the aims of the compilation, an introduction, abbreviations used therein, etc., and incorporating a preface by Francis

Quirin, President of the French Mycological Society. The next chapter tabulates the reagents, formulae, etc., which will be found in the pages which follow.

The largest part of the publication, 214 pages in all, brings together the reactions resulting from a specified, described and documented reagent when applied to a particular tissue or structure. For each case, where appropriate and possible, the same arrangement is used in the presentation so that direct comparisons can be made for the procedure and the reaction found in the same tissue in different reagents. Chapter 4 is particularly helpful because it deals with the application of selected chemicals to dried material, and in the following chapter discussion is focused on *Russula*, a genus in which chemicals have been used extensively in its systematics. The methods by which the reagents are made-up and applied are clearly given. Chapter 6 deals with naturally occurring pigments and their distribution within the sporomes. It is admirable that the author covers all the main groups of fungi when considering how to deal with the specimens in hand and their chemical characteristics.

A cross-referenced index follows, which is extremely helpful, especially as it cites the various species of fungi, or the genera which are mentioned under each reaction. The format for the whole publication parallels papers in *Documentes Mycologiques*, a journal in which Charbonnel regularly publishes. This is an excellent publication which will be of very great help to those undertaking this kind of work, and students should be encouraged to utilise the contents as the tests have a significant role to play in areas not attainable by so-called cutting edge science!

But for would-be readers, I suggest that on purchasing a copy it should be immediately rebound with a firmer spine, otherwise the pages will soon dismember.

Roy Watling
Caledonian Mycological Enterprises, Crelah,
26 Blinkbonny Avenue, Edinburgh EH4 3HU, UK

Frontiers in Basidiomycote Mycology. Edited by Reinhard Agerer, Meike Piepenbring & Paul Blanz. 2004. IHW Verlag, P.O. Box 119, D-85378 Eching, Germany (e-mail: dr.schmid@ihw-verlag.de). Pp. 430, figs 124, col. pl. 80. ISBN 3 930167 57 3. Price: 119 €.

Despite several requests to the publishers, no copy has been received. This implies that the publisher does not consider that the work merits coverage in *Mycotaxon.*

Cortinariales. By Vincentas Urbonas. 2005. UAB 'Valstiečių Laikraštis', Vilnius, Lithuania. [Available from Institute of Botany, Žaliųjų ežerų 49, LT-08406 Vilnius 21, Lithuania. Pp. 288, figs 118, col. plates 32. [Mycota Lithuaniae Vol. 8 (5).] ISBN 9986 847 85 0. Price: 28 €.

This work is part of a series of books dealing with the mycobiota of Lithuania. It is written in Lithuanian, but contains a seven page long English summary with keys to the included genera.

In this book, the *Cortinariales* are subdivided into two families, *Cortinariaceae* and *Crepidotaceae,* and in total 291 species belonging to 13 genera assigned to these families are described. Keys are provided for all included species, but they are difficult to use for people who do not know Lithuanian. The genera and numbers of species covered are: *Cortinarius* (124 spp.), *Crepidotus* (14), *Galerina* (27), *Gymnopilus* (12), *Hebeloma* (30), *Hebelomina* (1), *Inocybe* (65), *Leucocortinarius* (1), *Naucoria* (X), *Pellidiscus* (1), *Phaeocollybia* (3), *Rozites* (1), and *Simocybe* (4). *Cortinarius* is subdivided into five subgenera: *Cortinarius* (12), *Dermocybe* (12), *Myxacium* (10), *Phlegmacium* (37), and *Telamonia* (53). *Hebeloma* is divided into subgenera *Denudata* (18) and *Hebeloma* (12). *Galerina* is subdivided into subgenera *Phaeogalera* (24) and *Galerina* (3). And subgenera *Inocybe* (19) and *Inocybium* (46) are used for *Inocybe* species.

Basionyms, important synonyms, and iconography are provided for all included taxa. The nomenclature follows *Index Fungorum*, but is not always up to date. For example, *Rozites* is treated as a distinct genus, and the species epithet of *Cortinarius harcynicus* is given as '*hercynicus*'. Drawings of spores or other important microscopical structures are provided for selected taxa only, but they are more raw outlines than informative line drawings. An index of scientific names enables the reader to easily find all included taxa. The reference list includes a few interesting recent Lithuanian publications, but otherwise mainly includes publications up to the nineties; and more recent treatments (or iconography) of the included genera have not been considered in this work. The appendix of 32 colour plates provides either drawings or photographs of 163 of the species treated in the book. The colour drawings of the basidiomes are usually quite well done; but the quality of the photographs varies from quite good, to others which are out of focus or do not show the important features of the fungus. However, most pictures are supplemented by a line drawing.

In summary, this book is the first, well-investigated inventory of the cortinarioid fungi occurring in Lithuania. Those who understand the language will also find it a useful aid to the identification of fungi in Lithuania and surrounding countries, especially because pictures and line drawings are given in the same volume. Thus, it can be expected that with increasing sampling, the number of fungal species in this order known from Lithuania will rise. Consequently, in spite of a few shortcomings, this book will certainly help Lithuanian mycologists to study the brown-spored fungi of their beautiful country much more easily.

URSULA PEINTNER
Institute for Microbiology, Leopold Franzens University Innsbruck,
Technikerstrasse 25, A-6020 Innsbruck, Austria

Checklist of Polish Larger Basidiomycetes. By Władysław Wojewoda. 2005 ["2003"]. [Biodiversity of Poland Vol. 7.] W. Szafer Institute of Botany, Lubicz 46, PL–31–512 Kraków, Poland (e-mail: ed-office@ib-pan.krakow.pl). Pp. 812, fig. 1. ISBN 83 89648 09 1. Price: 58 €.

This is a welcome addition the literature of macromycetes of Europe. In the ninth edition of *Ainsworth & Bisby's Dictionary of the Fungi* (Kirk *et al.* 2001) works on the macromycetes of this continent were mentioned for Bulgaria, former Czechoslovakia, Denmark, France, Great Britain, Greece, Italy, Scandinavia, Spain, Sweden and Switzerland. However, Poland, the eighth largest country of Europe in area, was omitted.

The checklist of Polish larger basidiomycetes includes about 400 genera and 2650 species, although some of the listed names are invalid or otherwise contrary to the *Code*. The true number of nomenclaturally correct larger basidiomycete taxa known from Poland is actually about 2550.

Over 2100 species of agaricoid and boletoid fungi (Moser 1983) and about 2000 species of aphyllophoroid, gasteromycetoid, tremelloid, auricularoid, dacryomyceteoid and tulasnelloid fungi (Jülich 1984) are known in Europe. According to Wojewoda, these numbers are certainly too low and many more fungi occur in Europe. In the former German Federal Republic, 3150 species of basidiomycetes (Krieglsteiner 1991) were known through 1991, about a thousand more than the number known in Poland in 2003. Because Poland and the western part of Germany are comparable in terms of area, climate and nature preservation, a similar number of species might be expected to grow in Poland. In the years to come, Polish mycologists face the important task of finding these fungi.

Wojewoda includes data on threatened species compiled from the Red List of Threatened Macrofungi in Poland. To enable the data to be compared with information on threatened fungi in other European countries, categories of threat from the following countries are cited: Austria, Belgium, Bulgaria, Czech Republic, Estonia, Finland, Germany, Great Britain, The Netherlands, Lithuania, Latvia, Norway, Switzerland and Sweden.

The Polish names of the fungi given are derived from many authors of papers published between 1830 and 2003. The habitats in which the fungi grow, the plant community, substratum, mode of nutrition and fruiting time are detailed. Examples of species distribution in Poland, including the regions, form the most extensive part of the account of each species. For some very rare species, all or nearly all sites are given, while for fairly common species only selected sites are cited. For very common and common one (except a few like *Phallus impudicus* and *Langermannia gigantea*) no sites are given. Readers and all users of this new checklist can only congratulate the author on seeing the book published after 14 years of work.

Kirk, P.M., Connon, P.F., David, J.C., & Stalpers, J. (2001) *Ainsworth & Bisby's Dictionary of the Fungi*. 9th edn. Wallingford: CABI Publishing.

Moser, M. (1983) Die Röhrlinge und Blätterpilze (*Polyporales, Boletales, Agaricales, Russulales*). In: H. Gams (ed.) *Kleine Kryptogamenflora* II b/2: *Basidiomycetes*. 2. Teil 5., bearbeitete Aufl. VEB. Jena: G. Fischer.

Jülich, W. (1984) Die Nichtblätterpilze, Gallerpilze und Bauchpilze. *Aphyllophorales, Heterobasidiomycetes, Gasteromycetes*. In: H. Gams (ed.) Kleine Kryptogamenflora II b/1 *Basidiomyceten* I. Stuttgart: G. Fischer.

Krieglsteiner, G. J. (1991) Verbreitungsatlas der Grosspilze *Deutschland* (*West*.). Vol. 1. *Ständerpilze*. Teil A. *Nichtblätterpilze*. Teil B. *Blätterpilze*. Stuttgart: Verlag E. Eugen, Stuttgart, pp. 421–1016.

Bronislaw Zyska
Wolności Street 47/1, 41–500 Chorzów, Poland

Guía de los hongos de Alcalá de Henares (Macromicetes). By Michel Heykoop Fung-a-You & Rosa Antolín Bellver. 2005. Servicio de Publicaciones, Ayuntamiento de Alcalá de Henares, Concejalía de Medio Ambiente, Instituto de Planificación y Gestión Ambiental, Quinta de Cervantes, Calle Navarro y Ledesma 1, Alcalá de Henares, Madrid, Spain. Pp. 120, col. plates. ISBN not indicated (Dep. Legal: M-52838-2004). Price: Free while stocks lasts.

This amazing guide, completely financed by the local authority of Alcalá de Henares, the city where Cervantes lived part of his life in Spain, is an example of how local administrative bodies can promote interest in fungi, as a part of their broader concerns for nature and its conservation, and considering organisms as part of the area's natural heritage. It provides a concise history of mycological studies in the area, a catalogue that describes 65 species beautifully illustrated by coloured paintings rom the 200 known from Alcalá de Henares. It comprises seven chapters, some of which are brief introductions as to what a fungus is, habitats where they grow, fungal interactions with other organisms, the importance and applications of fungi, fungal classification, and characters of macromycete fruit bodies, as well as a key for the 65 species treated in the guide. Chapter 7 provides scientific and common names (where they exist), macroscopic descriptions, detailed drawings of the species (the second author, Michel's wife), and observations on their culinary or toxic properties, medicinal fungi, and fungi of industrial interest. A basic glossary of scientific terms is provided, as well as an index to the species. A three-week exhibition of the original paintings in a hall close to the town centre was also staged in April-May 2005. This is useful book for amateurs, and all interested in fungi from this area, combining identification with sound information on the broader relevance of fungi. The initiative in making such a work available free of charge is a model that I would like to see followed by many other local authorities worldwide.

Teresa Iturriaga
Departamento biología de Organismos, Universidad Simón Bolívar
Apartado 89000, Sartenejas, Baruta, Edo. Miranda, Venezuela

Palearctic *Lyophyllaceae* (*Tricholomatales*) in Northern and Eastern Europe and Asia. By Kuulo Kalamees. 2004. Estonian Academy Publishers, Tartu, Estonia. [Scripta Mycologica No. 18.] Pp. 135, figs 32, maps 3. ISBN 9985 50 356 2. Price: Not indicated.

This book presents a survey of the taxonomy, ecology and distribution for most of the genera in family *Lyophyllaceae sensu* Bon 1999 from the Palearctic regions of Europe and Asia: *Lyophyllum* s.str., *Hypsizygus*, *Gerhardtia*, *Calocybe s. str.*, *Tricholomella*, *Rugosomyces*, and *Asterophora* are treated in detail but the genus *Tephrocybe* is not considered. The book is divided in two parts : an introductory part (pp. 5-15) and a taxonomic part (pp. 16-105). The introductory part summarizes the actual state of knowledge on family *Lyophyllaceae*, presents the significant taxonomic features used for species determination, and the classification and the abbreviations used by the author.

The taxonomic part constitutes some kind of practical guide to the identification of the Palearctic *Lyophyllaceae*, which is certainly not one of the easy agaric families as pointed out by the author. The author provides a key to genera and species, species descriptions illustrated by some line drawings of microscopical features (spores, basidia and mycelium), clarifies differences in species concepts between mycologists, and adds also valuable information about the distribution and ecology of the species. Ecology is indeed very important but too often a neglected aspect in many revisions. Particularly in the case of *Lyophyllaceae*, ecology may very well be one of the most important factors underlying evolution since molecular studies have shown that all the classifications proposed for the tribe *Lyophylleae* are artificial and that species sharing a mode of nutrition cluster together.

The book is well edited and provides detailed macroscopic and microscopic descriptions and well referenced discussions and comments. It is therefore all the more deplorable that the quality of the illustrations is disappointing. The rather simplistic line drawings often lack sufficient precision or detail (e.g. scales are not always respected, as for the spores of *L. loricatum*), while in others being superfluous or not essential for understanding or identification or not representative of the text (e.g. *Rugosomyces obscurissimus* spores). The reader has the impression that the author considers microscopic illustrations of secondary or no importance (even when a lot of collections have been checked, as for example in the case of *L. decastes*, where one would expect mature basidia instead of basidioles). As already very few morphological characters are available to separate species in virtually all of the *Lyophyllaceae*, the illustration of taxonomically significant microscopic features such as size, shape and ornamentation of spores should be as detailed, precise and representative as possible. In my opinion, the book would also have benefited from coloured pictures illustrating the species (in the form of an annex for example) in addition to a list of often too inaccessible references to most of the users of this type of monograph.

I also regret that the discouraging nomenclatural chaos in *Tephrocybe* was apparently responsible for its exclusion from this revision of *Lyophyllaceae*. Indeed, molecular studies suggest that both *Tephrocybe* and *Lyophyllum* are polyphyletic. Exclusion of either one results automatically in partial revisions of the species in several of the natural groups within *Lyophyllaceae*. In that sense, the impact of this study is reduced, not only as a taxonomic guide to *Lyophyllaceae*, but also as a useful tool for future revisions of the more natural entities.

With the new insights of molecular systematics, a reappraisal of the morphology of *Lyophylleae* in the context of a more natural classification of this fungal group will hopefully soon emerge. It will facilitate the difficult task of the purely morphological approach in *Lyophylleae*. Taxonomic studies as this one are really needed to help clarify the nomenclature, morphology and ecology of this very difficult group of fungi, and this book can therefore be considered as an important contribution to attaining this goal.

VALERIE HOFSTETTER
Verdonnet 32, 1010 Lausanne, Switzerland

Monograph of the genus *Hemileia* (*Uredinales*). By Anja Ritschel. 2005. J. Cramer in der Gebrüder Borntraeger Verlagsbuchhandlung, D-14129 Berlin, Germany. [Bibliotheca Mycologica No. 200.] Pp. 132, pl. 8, figs 39. ISBN 2 443 59102 7. Price: 44 €.

The work on this tropical fungal genus, known essentially for its parasitism on coffee, but affecting at least ten families of angiosperms, has been conducted on herbarium material, mostly from European herbaria, but also from ones in the USA and South Africa. In many cases the descriptions rely on (very) old material, which is probably caused by the absence of collectors, providing fresh samples, in the tropics. For some species, no material was available to the writer. Descriptions were then gathered from previous publications.

There is a pertinent discussion of the validity of the genus name and the affiliation of *Hemileia* with the *Chaconiaceae*. The author keeps all species without a known teleomorph in the anamorph genus *Uredo*. This concerns 17 of the 43 species described. A new genus, *Desmosurus* is proposed. One new name, a new species, and some new combinations are introduced. Doubtful and excluded taxa are mentioned.

Full attention is given to the formation of the sori, and two types, suprastomatal or erumpent, are distinguished. The individual species descriptions are as complete as can be, and include all the references needed. Urediniospore characters are congruent with the host range and differentiate the species, and the author gives a key based on length and echinulation of the urediniospores. For the species of *Hemileia s. str.*, a description of the teliospores is provided. The drawings of spores are very good and clear, and the SEM photographs excellent.

The cited literature comprises 125 references. The last cytological study mentioned is dated 1968, and two references concern molecular techniques, which were probably not possible with the herbarium material available. Further work remains to be done in that field.

This book is a valuable contribution to our knowledge of the genus, and will be of great use for mycologists everywhere, and not only in the tropics. One species is described from Japan, and another reported from orchids in heated greenhouses in Europe.

ARTHUR VANDERWEYEN
Avenue Cardinal Micara 9, B-1160 Bruxelles, Belgium

***Russulaceae*: *Lactarius*.** By R[onald] W. Rayner, assisted by R[oy] Watling & E[lizabeth] Turnbull. 2005. Royal Botanic Garden, 20A Inverleith Row, Edinburgh EH3 5LR, UK (e-mail: pps@rbge.org.uk). [British Fungus Flora Vol. 9.] Pp, 203, figs 200. ISBN 1 872291 34 1. Price: £12.50

This ninth number in the agarics and boleti series documenting the British fungi treats *Lactarius* in the Russulaceae. It is presumed that Roy Watling and Elizabeth Turnbull brought the compilation together for publication after the passing of “Ronnie” Rayner, although this is not mentioned in the publication. An item that appeared early on in the series and is included with this number is that of the trifold colour identification chart.

This chart will be repeatedly consulted for determination of spore colour in mass as well as other colours described for the basidiomes.

The overall treatment of *Lactarius* follows the same general scheme presented in previous issues of this series with introductory text, references, family description, a generic key to just *Russula* and *Lactarius* with a note that sequestrate representatives are to be keyed elsewhere. *Lactarius* is then treated in detail with a description and discussion of features (including useful macrochemical reagents) in the genus followed by a key to seven sections, diagnostic synopses of the sections, the systematic arrangement (essentially that of Singer's fourth edition with some minor rearrangement, and incorporating Hesler and Smith's treatment of sect. *Tristes*). This is followed by a complete dichotomous key to the 64 species. A handful of varieties are also treated. Although key to sections is provided, a key to species in each section (and subordinate subtaxa) is lacking.

The individual descriptions of species occupies the bulk of the publication and these are quite complete with attention to the details of fresh macroscopic appearance, macrochemical reactions, and important microscopical features, describing spores, hymenial cystidia and the pileus surface. The ecological habitat, phenology and distribution in Britain follow the description along with a commentary on distinctive or odd features and comparisons to like taxa. On a rare occasion, mention is made of the taxon as it might occur outside of Britain. In addition, some general synonymy is given along with citation of illustrations (icones) representing well the British species.

The descriptions are followed by a listing of species according to ecological preference such as: in oak woods, with ash, with *Betula nana*, on calcareous soils to name just a few. Some species are mentioned more than once in this list. There are further lists of synonyms and misidentifications, rejected names (and why rejected), an index to epithets mentioned in the commentaries, and lastly the index to described species. The last 65 pages of 203 are given over to line illustrations of habit sketches and microscopic features of spores, cystidia, and hyphal configurations making up the pileus surface. These latter are relevant in a diagnostic sense as examples of taxa in particular sections.

It is noteworthy to mention that the descriptions are almost entirely based on observation of fresh material, either collected by the author or given to him by several individuals who are dutifully acknowledged.

For those who need to identify *Lactarius* species among the macromycetes in Britain, users will find this treatment comfortably familiar to past treatments in the series as well as thorough and well documented. The addition of the colour chart will be necessary for colour determination but is also an added bonus and can be used with the previous publications.

ROY E. HALLING
Institute of Systematic Botany
The New York Botanical Garden
Bronx, NY 10458-5126, USA

Ascomycetes

Discomycetes. Fasc. 1. Familiae *Ascobolaceae* (species coprotrophicae), *Iodophanaceae* (species coprotrophicae), *Ascodesmidaceae* (species coprotrophicae), *Pezizaceae* (species coprotrophicae), *Pyrenomycetaceae* (species coprotrophicae), *Thelebolaceae* (species coprotrophicae). By V. P. Prochorov. 2004. Oficina Editoria KMK, 123100 Moscow a/r 16, Russia (e-mail: kmk2000@online.ru). Pp. 256, figs 148. [Definitorium Fungorum Rossiae.] ISBN 5 87317 136 X. [In Russian.] Price: not indicated.

This volume aims to provide a detailed account of the coprophilous pezizalean discomycetes known from Russia. It treats 194 species disposed through 23 genera: *Ascobolus, Ascodesmis, Ascozonus, Caccobius, Cheilymenia, Coprobia, Coprobolus, Coprotus, Dictyocoprotus, Dictyosporus, Fimaria, Hapsidomyces, Iodophanus, Lasiobolus, Ochotrichobolus, Peziza, Pseudacozonus, Ramgea, Saccobolus, Thecotheus, Thelebolus, Trichobolus,* and *Trichophaeopsis.* These are all listed here as only 11 of the genera are named on the contents page. For each species, the place of publication of the accepted name and any synonyms is provided (sometimes with a page-range not the actual page). Descriptions of genera and species are detailed, keys are provided, there are references to published illustrations, and especially welcome fine original line illustrations of microscopic features -- covering three quarters of the included species. Notes on habitat and frequency are very brief, and regions in Russia the species occur are sometimes indicated, but not consistently; extra-Russian distributions are also summarized. The work has evidently been in press for some years, as the latest reference cited is one of the author's from 1996 and so seems a little dated in part, for example in not taking up *Pseudombrophila* for the later synonym *Fimaria.* While this is no rival to Doveri's *Fungi Fimicoli Italici* (2004; see *Mycotaxon* **90**(1): 220-221, 2004), it is a major achievement for a mycologist who has only been publishing on these fungi since 1991, and does include some species not treated in the Italian account. I will certainly find the illustrations of value in trying to name minute coprophilous discomycetes.

Lichen-forming fungi

Lichens: An Illustrated Guide to the British and Irish Species. By Frank S. Dobson. 2005. 5th edn. Richmond Publishing, P.O. Box 963, Slough SL2 3RS, UK (e-mail: rpc@richmond.co.uk). Pp. 480, col. pls, maps. ISBN 0 85546 095 4 (hardback), 0 85546 096 2 (paperback). Price: £ 45 (hardback), £ 35 (paperback).

Each fresh edition of this book, the first of which was published in 1979, goes from strength to strength. The number of species treated has increased from about 450 in 1979, to about 700 in the fourth edition of 2000 (reviewed in *Mycotaxon* **78**: 510, 2001), to about 850 in the fifth – approaching half of all the 1800 or so lichen-fungi known from Great Britain and Ireland. Further, an increasing number are illustrated in colour and crisper and more true-to-life than ever thanks to the use of digital photography. The tried and tested format of keys that has been used so successfully by almost a new generation of amateur lichenologists has been maintained, as has the basic layout of the entries with the neat and now familiar distribution maps based on data in the British Lichen Society's Mapping Scheme. Unfortunately, the nomenclature follows the last British checklist too slavishly, for example in the adoption of some of the segregates proposed for parmelioid lichens for the first time after papers synonymizing several had appeared, retaining *Usnea subfloridana* as distinct from *U. florida,* and not taking up *Xanthoria aureola* for *X. ectaneoides.* In consequence there will be some discrepancies from the new edition of the multi-authored *The Lichen Flora of Great Britain and Ireland* currently in preparation. But these are small points. To produce one identification guide is a major achievement for an amateur mycologist, but to be able to produce one in five continuously improving editions over a period of 26 years is an unparalleled event and a 'first' for lichenology. The impact that this title has had on developing expertise in lichenology in Great Britain and Ireland over this extended period of time has been immense, especially through its use on several field courses each year by its' author and other tutors. Lichenology owes Frank an immense debt.

Opredelitel' Lishainikov Rossii. Vol. 8. ***Bacidiaceae, Catillariaceae, Lecanoraceae, Mycobilimbiaceae, Rhizocarpaceae, Trapeliaceae.*** By M. P. Andreev, L. I. Bredkina, N. S. Golubkova, A. A. Dobrysh, Y. V. Kotlov, I. I. Makarova, I. N. Urbanavichene & G. P. Urbanavichus. 2003. Nauka, St Petersburg, Russia. Pp. 278, figs 93. ISBN 5 02 026044 4. Price: Not indicated.

Opredelitel' Lishainikov Rossii. Vol. 9. ***Fuscideacae, Teloschistaceae***. By A. Y. Khododovtsev, S. Y. Kondratyuk, I. I. Makarova, & A. N. Oxner. 2004. Nauka, St Petersburg, Russia. Pp. 340, figs 133. ISBN 5 02 026207 2. Price: Not indicated.

This series started publication in 1971 as *Opredelitel' Lishainikov SSSR*, with 'SSR' being replaced by 'Rossii' from volume six which appeared in 1996. I was pleased to see these two recent volumes as no additions to the series had appeared since volume seven in 1998, and it would have been unfortunate if this ambitious project joined the ranks of unfinished multi-volume projects. The style follows that of the previous volumes with keys and detailed descriptions, but with more line and half-tone illustrations.

Volume Eight tackles a series of difficult families in which concepts are still being worked out and in many cases await testing by molecular phylogenetic approaches. But, at least the authors have endeavoured to follow the latest proposals, for example in accepting *Calvitimela* (into which three new combinations are made). In some cases all the species in a genus are not treated, for instance under *Lecanora* only the *L. marginata/ L. sulphurea* complex is covered. Volume Nine has been particularly awaited for its treatment of *Caloplaca* and especially *Xanthoria* on which Sergey Kondratyuk and the late A. N. Oxner worked for many years. The treatment of *Caloplaca* follows traditional lines, and the account is particularly well-illustrated by line drawings. The authors' approach to *Teloschistes* and *Xanthoria,* however is much more controversial with the introduction of three new genera, none validly published here: *Oxneria* for the *X. fallax* group (18 spp.), *Rusavskia* for the *X. elegans* group (11), and *Xanthoanaptychia* for the *Teloschistes chrysophthalmus* group (5). Further, many more species are being accepted in these segregate genera than are currently in use. There may be good molecular evidence for such changes, but it would have been preferable to see the new evidence first and for the scientific names and combinations to have been validly published elsewhere before being utilized in an essentially floristic study.

As the series grows, it becomes increasingly difficult to check in which volume a particular taxon is treated, and it would be great if in future that each volume had a complete list of genera so far treated in the series with an indication in which volume they were to be found. Treated families were listed in each part up to and including volume six, which was a gesture in this direction, but even that was dropped in subsequent volumes.

The Lichens, Lichenicolous and allied Fungi of Poland: An annotated checklist. By Wiesław Fałtynowicz. 2003. W. Szafer Institute of Botany, Polish Academy of Sciences, Lubicz 46, 31-512 Kraków, Poland (e-mail: ed-office@ib-pan.krakow.pl). Pp. 435, figs 1. [Biodiversity of Poland Vol. 6.] ISBN 83 89648 06 7. Price: Not indicated.

The tradition in which Polish records are synthesized is not only maintained but taken to new heights in this new checklist. The previous checklist (Fałtynowicz 1993) included 1500 lichenized and 119 lichenicolous fungi, and these totals have now swelled to 1554 and 214 respectively. But the 1993 list consisted only of the scientific names, with no references as to sources let alone information on habitats and distribution. This one has synonyms listed under the names, information on ecology and localities (down to province), and literature references from the mid-nineteenth century on. Each genus and species is also given a name in Polish, most coined here for the first time. But I have to wonder if this is really worthwhile – especially in the case of the lichenicolous fungi so named! This is a landmark publication of synthesis on the Polish lichens and lichenicolous fungi, on which the author has worked for over 25 years, and is the most detailed checklist I have seen for some years for any country.

Fałtynowicz, W. (1993) A checklist of Polish lichen forming and lichenicolous fungi including parasitic and saprophytic fungi occurring on lichens. *Polish Botanical Studies* **6**: 1-65.

Flora Liquenológica Ibérica: *Ostropales*: *Graphidaceae, Solorinellaceae; Gyalectales: Gyalectaceae.* 2004. Sociedad Española de Liquenología, Murcia, Spain. Pp. 48, figs 9, map 1. ISSN 1696-0521. Price: 10 €.

The first volume in this series dealt with the *Peltigerales* (see *Mycotaxon* **92**: 492-493, 2004). This second volume covers somewhat disparate groups by different authors, but is presented to the same high standards in both the descriptions and drawings. The plate of ascospores in *Gyalecta* is especially fine. As the series builds it will become of increasing value as a major work of reference.

Catalogue of the lichenized and lichenicolous fungi in Bulgaria. By Helmut Mayrhofer, Cvetomir M. Denchev, Dimitar Y. Stoykov & Siyka O. Nikolova. 2005. Bulgarian Mycological Society, c/o Institute of Botany, Bulgarian Academy of Sciences, 23 Acad. G. Bonchev Street, 1113 Sofia, Bulgaria (e-mail: denchev@bio.bas.bg). Pp. 59. [Mycologica Balcanica **2**: 3-61.] ISSN 1312-3300. Price: 19 €.

This paper is mentioned here as it was specifically submitted to *Mycotaxon* for review. It is the first synthesis of information on the lichens of Bulgaria to have been published since the flora of Popnikolov & Zhelezova (1964). That work accepted 636 species and included keys, descriptions, and many illustrations. This new checklist has almost 50% more species, 893, of which a mere nine are lichenicolous and 14 non-lichenized fungi traditionally studied by lichenologists. The list is based on 216 publications supplemented by previously unpublished reports backed by herbarium material. Eighteen species are reported from Bulgaria for the first time; some of the latter are rather widespread in Europe (e.g. *Lecanora saligna, Phylctis argena, Tuckermannopsis chlorophylla*) and their inclusion at such a late date is a witness to the scant attention lichenology in the country has received. Species are arranged alphabetically and their sources are given, but without any information on substratum or locality except in the case of new records for the country. An impressive list of synonyms is included, with 1625 infrageneric epithets. Hopefully, the availability of this new work will stimulate interest in lichenology in Bulgaria.

Popnikolov, A. & Zhelezova, B. (1964) *Lishei.* [Flora na B"lgariya.] Sofia: Narodna Prosveta.

101 Common Mosses, Liverworts & Lichens of the Olympic Peninsula. By Martin Hutten, Karen Hutten & Andrea Woodward. [2001]. [US Government Printing Office.] Pp. xi + 109, col. ISBN none. Price: US $ 9 [now out of print].

This delightful little spiral bound book, only 14.5 x 11.5 cm so it easily slips into a pocket, seems to have escaped other listings of lichen literature. Bryophytes and lichens are conspicuous even to the casual tourist in the high-rainfall parts of the Olympic Peninsula in Washington State, USA. The annual rainfall is said to be higher in some parts of the Peninsula than anywhere else in North America (600 cm p.a. plus), and pendent lichens to over 2 m in length can be encountered. After the introductory matter, each species has a full page almost half of which is a high-quality colour photograph, and the remainder presents descriptive notes, information on size, and separation from similar species. Altitudinal ranges are indicated by a vertical scale bar, and the species are grouped by habitat (e.g. forest floor, log, conifer). In many case there are small inset photographs of allied species that might be confused with those featured. The lichen pages have been prepared to the highest standards, with critical material checked by Bruce McCune. The production of the book was funded by several organizations including Cannon, The Green Earth Campaign, National Park Service, US Geological Survey, National Park Foundation, and the Northwest Interpretive Association; their vision in supporting such an initiative to generate a low-price product is to be commended. I found the little book very valuable in the field when encountering some of the endemic *Pseudocyphellaria*'s for my first time on the Peninsula in October 2005, but after praising, recommending it, and being presented with a copy by Maggie Rogers (Portland, Oregon), it emerged that this little gem was already out of print. If you see one available second-hand do secure it, even if just to sell one at a society auction.

Conidial fungi

***Sporidesmium, Endophragmiella* and related Genera from China.** By Wenping Wu & Wenying Zhuang. 2005. Fungal Diversity Press, Centre for Research in Fungal Diversity, Department of Ecology and Biodiversity, University of Hong Kong, Pokfulham Road, Hong Kong SAR, People's Republic of China. Pp. x + 351, figs 152. [Fungal Diversity Research Series no. 15.] ISBN 962 86765 8 X. Price: US $ 80.

The fungi formerly referred to *Sporidesmium* have been segregated into an increasing number of genera over the course of the last three decades. The generic separations have been based on methods of conidiogenesis and conidial secession, schizolytic (*Sporidesmium*-type) or rhexolytic (*Endophragmiella*-type), and then by the septation in the conidia, which can be eu- or distoseptate, or the form of the conidia. These fungi had been little studied in China, indeed only one species of *Sporidesmium* is mentioned in Teng (1996), *S. polymorphum* (a record apparently overlooked by the authors), but Wu made around 300 collections in 'the last few years'. This book is a report of those collections and does not include studies of material in HMAS (Beijing) or other Chinese collections. This makes the results all the more impressive, as out of those collections emerged 143 species in 25 genera, including one genus and 43 species new to science (i.e. 30 % of those collected), and 16 new combinations. Further, 23 of the genera and 99 species proved to be first records for China.

The introduction includes comparative illustrations of the differences between the genera, as well as a key, which is sure to be of value to all starting to get to grips with the current generic concepts in the group. Very full descriptions are provided and there are clear line drawings, mostly full-page, but no photographs. Photographs would have been a valuable addition as it is not always clear what artistic licence has been taken in adapting complex wall structures to drawings. In particular, incomplete septa are in some cases illustrated between thickened distoseptate cells, with structures indicated between some and not others. That may well be so, but photographs would have made the situation incontrovertible. Also, there are strong similarities between the conidia of some species placed in different genera, for instance the *S. novozymium* group and *Ellisembia bambusae*, which even have similar gelatinous sheaths at their apices, and I wonder how significant a complete as opposed to an incomplete septum really is. It would be interesting to see a molecular phylogenetic study carried out on these collections to address such issues.

Original places of publication are always given, but there is no indication as to whether the type collections of already described species were examined. At least in some cases the concepts seem to have been taken from publications of other authors. With respect to the type specimens and ex-type cultures of the newly described species, I was somewhat concerned to read that all the specimens studied including the types "are preserved in Wu's mycological herbarium in Novozymes, China", with living cultures in the Novozymes collection. This is hardly in the spirit of the *Code*, and at least the holotypes would have been better deposited in HMAS (Beijing).

Notwithstanding these concerns, there is no question that this work represents a substantial contribution to our knowledge of these fascinating fungi, and that it has been carried out with care and patience that is in the best traditions of studies of dematiaceous hyphomycetes. With so many taxa covered, this work is set to become the major international reference work on *Sporidesmium* and similar genera for many years to come.

Teng, S. C. (1996) *Fungi of China.* Ithaca, NY: Mycotaxon.

Morphotaxonomic Revision of Fungicolous *Cladosporium* Species. By Bettina Heuchert, Uwe Braun & Konstanze Schubert. 2005. Druckerei der Martin-Luther-Universität Halle-Wittenberg, Kröllwitzer Straβe 44, D-06120 Halle/Salle, Germany. Pp. 78, figs 26, plates 2. [Schlechtendalia Vol. 13.] ISSN 1436-2317. Price: 5 €.

With the nature of *Cladosporium s. str.* now clarified, the numerous species described but whose affinities have not been reassessed in the light of current concepts need to be revisited. Here, this task is undertaken for the fungicolous (including the lichenicolous) species. Most fungicolous species prove to belong to *Cladosporium s. str.* Eleven obligately fungicolous species are accepted and described in detail, and illustrated by line drawings; information on hosts, geographical occurrences, and lists of specimens examined is also provided. One of these species is newly described (*C. gerwasiae* on a *Gerwasia* sp. rust on *Rubus*), and a new variety of *C. exobasidiae* occurring on *Exobasidium vaccinii* is also recognized . In addition, descriptions of six saprobic species which can also occur on decaying fungi are treated in similar detail. Twelve species are regarded as dubious or doubtful due to insufficient data or ambiguous information as to their substrate, and seven are excluded from the genus. New generic names are introduced for two of the excluded species, *Digitopodium* (for *C. hemileiae*) and *Parapericoniella* (for *C. asterinae*), and there are suggestions that two more may eventually also require different generic names.

The authors place square brackets ([. . .]) around author citations for infraspecific taxa other than the type variety, i.e. *C. exobasidii* Japp var. *exobasidii* but *C. exobasidii* [Jaap] var. *verruculosum* Heuckert *et al.* Under the *Code,* the citation of the author of the species name is not appropriate in the last and similar cases. However, if it is considered desirable to mention the author of the species name, their placement between squared brackets is a novel approach, though potentially ambiguous, as the device has a long informal use for the indication of pre-Linnean names.

This is a morphologically based study in which especial attention has been paid to the nature of the conidial scars and conidiophore branching, and there are coloured plates of the type species of the new genera and scanning electron micrographs of scars. Keys by the features of the fungi alone, and also by host, are provided. This is a carefully executed study in the best traditions of hyphomycete taxonomy and will facilitate and encourage future critical identifications in this ecological group of the genus.

Miscellaneous

Species Plantarum 250 Years: Proceedings of the Species Plantarum Symposium held in Uppsala August 22-24, 2003. Edited by Inga Hedberg. 2005. Uppsala University Library, Box 510, SE-751 20 Uppsala, Sweden (e-mail: acta@ub.uu.se). Pp. 219. [Symbolae Botanicae Upsalienses Vol. 33 (3).] ISBN 91 554 6192 1. Price: 215 SEK.

Although only one of 20 contributions in this work has a fungal (actually lichen) flavour through its examples, attention is drawn to it here as these include important background information to the Linnaean literature and Linnaeus' way of working -- as well as some thought-provoking contributions on the future of botanical nomenclature. The contributors include the leading authorities on Linnaeus (e.g. Charlie Jarvis, Bengt Jonsell, Per Magnus Jørgensen, Walter Lack) and others at the cusp of heated debates on aspects of the future of biological nomenclature (e.g. Werner Greuter, Kevin de Queiroz). A final tranch of seven papers deals with inventorying the world's flora, and occasions reflection on the huge gap between the state of plant and fungal inventories.

Biological Resource Centres and the Use of Microbes. Edited by Nelson Lima & David Smith. 2003. Micoteca da Universidade do Minho, Centro de Engenharia Biológica, Campus de Gualtar, 4710-057 Braga, Portugal. Pp. 422. ISBN 972 97916 3 5. Price: 30 €.

This volume represents the proceedings of the 22nd European Culture Collections' Organization (ECCO) Meeting held in Braga, Portugal, on 17-19 September 2003. It is important that those involved in the curation and maintenance of genetic resource centres of fungus cultures keep abreast of both technical advances in preservation science and the increasingly complex worlds of databasing and legal or ethical issues. Such occasions facilitate such exchanges of views with those working with other organisms, from bacteria to human cell lines. Starting with a powerful opening contribution on the importance of *ex situ* collections by Manuel Mota, the papers presented in four symposia follow. These concern: The use of microbes, Microbial identification techniques, Bioactive molecules, and Biological resource centres. There are also reports from a round-table discussion covering the Global Biodiversity Information Facility (GBIF), activities of the World Federation for Culture Collections (WFCC), Accredited Biological Resource Centres, and the realm of acronyms. If you curate a collection of fungal cultures and would like to obtain a copy, prompt action is recommended as only 300 copies of the proceedings were printed.

Nomenclatural novelties proposed in Mycotaxon 94

Anthracoidea setosae L. Guo, p. 47
Anthracoidea xizangensis L. Guo, p. 48
Blumenavia toribiotalpaensis Vargas-Rodriguez, p. 8
Clavulinopsis antillarum (Pat.) García-Sandoval & Cifuentes, p. 277
Coltricia tsugicola Y.C. Dai & B.K. Cui, p. 342
Cordyceps spegazzinii M.S. Torres, J.F. White & J.F. Bischoff, p. 257
Dactylellina illaqueata, D.S. Yang & M.H. Mo, p. 215
Fomitiporia torreyae Y.C. Dai & B.K. Cui, p. 344
Hirsutella crinita Z.Q. Liang, p. 352
Hirsutella hunanensis Z.Q. Liang, p. 351
Hirsutella zhangjiajiensis Z.Q. Liang & A.Y. Liu, p. 350
Hymenochaete flexosetosa Parmasto, p. 199
Hymenochaete geniculata Parmasto, p. 201
Hymenochaete legeri Parmasto, p. 203
Hymenochaete nothofagicola Parmasto, p. 212
Hypocenomyce isidiosa Elix, p. 219
Hypoxylon kanchanapisekii Suwannasai, Rodtong, Thienhirun & Whalley, p. 307
Hypoxylon sublenormandii Suwannasai, Rodtong, Thienhirun & Whalley, p. 307
Hypoxylon suranareei Suwannasai, Rodtong, Thienhirun & Whalley, p. 308
Lecanicillium pissodis Kope & Leal, p. 334
Lepiota catenariocystidiata Han C. Wang & Zhu L. Yang, p. 52
Lepiota trichroma Montoya & Bandala, p. 112
Lepraria lobata Elix & Kalb, p. 220
Leprocaulon australasicum Elix, p. 221
Lewia chlamidosporiformans B.S. Vieira & R.W. Barreto, p. 246
Otidea crassa W.Y. Zhuang, p. 366
Otidea onotica var. brevispora W.Y. Zhuang, p. 368
Paecilomyces parvosporus Y.F. Han & Z.Q. Liang, p. 360
Pertusaria cyathicola Elix, p. 223
Pertusaria subarida A.W. Archer & Elix, p. 134
Ramaria luteoaeruginea P. Zhang & Zhu L. Yang, p. 236
Ramaria pallidolilacina P. Zhang & Z.W. Ge, p. 238
Russula mukteshwarica K. Das, S.L. Mill., J.R. Sharma & R.P. Bhatt, p. 86
Streptopodium passiflorae (Syd.) Liberato & R.W. Barreto, p. 90
Thyrostoma negundinis (Berk. & M.A. Curtis) A.W. Ramaley, p. 131
Tuber umbilicatum Juan Chen & P.G. Liu, p. 2
Vamsapriya Gawas & Bhat, p. 150
Vamsapriya indica Gawas & Bhat, p. 150
Weddellomyces turcicus Halici & Orange, p. 249

Author Index—Volume 94

Aksoy, Ahmet, see **Halici & al.**

Archer, Alan W. & John A. Elix. A new species of *Pertusaria* from Western Australia. 94: 133–135. 2005 (2006).

Bandala, Victor M., see **Montoya & Bandala**

Barreto, R. W., see **Liberato & Barreto**

Barreto, Robert W., see **Vieira & Barreto**

Bhat, Darbhe Jayarama, see **Gawas & Bhat**

Bhatt, R.P., see Das & al.

Bischoff, Joseph F., see **Torres & al.**

Blanco, M.N., see **Checa & Blanco**

Boddy, Lynne. Book review: Biodiversity of Fungi: Their Role in Human Life [Deshmukh & Rai 2005]. 94: 371–372. 2005 (2006).

Bonuso, Enrico, see **Iotti & al.**

Breuil, C., see **Masuya & al.**

Checa, Julia & M.N. Blanco. Some interesting pyrenomycetous fungi on bark of *Quercus* spp. from Spain. 94: 225–230. 2005 (2006).

Chen, Juan, Pei-Gui Liu & Yun Wang. *Tuber umbilicatum*, a new species from China, with a key to the spinose-reticulate spored *Tuber* species. 94: 1–6. 2005 (2006).

Chen, Weimin, see **Yang & al.**

Chu, Huali, see **Han & el.**

Cifuentes, Joaquín, see **García-Sandoval & al.**

Constantinescu, Ovidiu, Vadim A. Mel'nik & Gerard J.M. Verkley. Two parasitic fungi on a new host, *Syringa* (*Oleaceae*). 94: 175–179. 2005 (2006).

Cui, Bao-Kai, see **Dai & Cui**

Dai, Yu-Cheng & Bao-Kai Cui. Two new species of *Hymenochaetaceae* from eastern China. 94: 341-347. 2005 (2006).

Das, K., S.L. Miller, J.R. Sharma, P. Sharma, & R.P. Bhatt. *Russula* in Himalaya 1: A new species of subgenus *Amoenula*. 94: 84-88. 2005 (2006).

De Luna, Efraín, see **García-Sandoval & al.**

Delivorias, P., see Z. Gonou-Zagou

Deng, H., & Y. -J. Yao. *Tricholoma equestre,* the correct name for *T. flavovirens* (*Agaricales*). Mycotaxon 94: 325–329. 2005 (2006).

Elix, John A. New species of sterile crustose lichens from Australasia. 94: 219–224. 2005 (2006).

Elix, John A., see **Archer & Elix**

Estrada-Torres, Arturo, see **García-Sandoval & al.**

Fan, Meizhen, see **Huang & al.**

García-Sandoval, Ricardo, Joaquín Cifuentes, Efraín De Luna, Arturo Estrada-Torres & Margarita Villegas. A phylogeny of *Ramariopsis* and allied taxa. 94: 265–292. 2005 (2006).

Gawas, Puja & Darbhe Jayarama Bhat. *Vamsaprija indica* gen. et sp. nov., a bambusicolous, synnematous fungus from India. 94: 149–154. 2005 (2006).

Ge, Zai-Wei, see **Zhang & al.**

Gilbertson, Robert L., see **Parmasto & Gilbertson**

Gonou-Zagou, Z., & P. Delivorias. Studies on Basidiomycetes in Greece 1: The genus *Crepidotus*. 94: 15–42. 2005 (2006).

Grand, L.F., & C.S. Vernia. Biogeography and hosts of poroid wood decay fungi in North Carolina: species of *Fomes, Fomitopsis, Fomitella* and *Ganoderma.* 94: 231-234. 2005 (2006).

Guo, Lin. Two new species of *Anthracoidea* (*Ustilaginales*) from China. 94: 47–50. 2005(2006).

Halici, M. Gökhan, Alan Orange & Ahmet Aksoy. *Weddellomyces turcicus,* a new species on a grey *Acarospora* from Turkey. 94: 249–252. 2005 (2006).

Halling, Roy. Book review: *Russulaceae: Lactarius* [Rayner et al. 2005]. 94: 383–384. 2005 (2006).

Han, Yanfeng, see **Liang & al.**

Han, Yanfeng, Zongqi Liang, Huali Chu & Jichuan Kang. *Paecilomyces parvosporus,* a new species with its relatives from Yunnan Province, China. 94: 357–363. 2005 (2006).

Hawksworth, David L. (ed.). Book reviews and notices. 94: 371–391. 2005 (2006).

Hodge, Kathie T., see **Huang & al.**

Hodge, Kathie T., see **Huang & al.**

Hofstetter, Valerie. Book review: Palearctic *Lyophyllaceae (Tricholomatales)* in Northern and Eastern Europe and Asia [Kalamees 2004]. 94: 381–382.

Huang, Bo, Chunru Li, Richard A. Humber, Kathie T. Hodge, Meizhen Fan & Zengzhi Li. Molecular evidence for the taxonomic status of *Metarhizium taii* and its teleomorph, *Cordyceps taii* (*Hypocreales, Clavicipitaceae*). 94: 137-147. 2005 (2006).

Huang, Bo, Richard A. Humber, Shigui Li, Zengzhi Li & Kathie T. Hodge. 2005 (2006). Further notes on the molecular taxonomy of *Metarhizium.* 94: 181–187.

Huang, Jianzhong, see **Liang & al.**

Huang, Ying, see **Yang & al.**

Humber, Richard A., see **Huang & al.**

Humber, Richard A., see **Huang & al.**

Hüseyın, Elşad, Faruk Selçuk & Ahmet Şahın. The world's second record of *Neoheteroceras flageoletii* reported from Turkey. 94: 94: 241-244. 2005 (2006).

Iotti, Mirco, Mauro Marchetti, Enrico Bonuso & Alessandra Zambonelli. Morphological and molecular characterization of the mycorrhizas of *Inocybe rufuloides* and *I. splendens.* 94: 75–84. 2005 (2006).

Iturriaga, Teresa. Book review: Guía de los hongos de Alcalá de Henares (Macromicetes) [Heykoop & Bellver 2005]. 94: 381. 2005 (2006).

Jiang, Y. & Y-J. Yao. ITS sequence analysis and ascomatal development of *Pseudogymnoascus roseus.* 94: 55–73. 2005 (2006).

Kaneko, S., see **Masuya & al.**

Kang, Jichuan, see **Han & el.**

Kim, G.-H., see **Masuya & al.**

Kim, J.-J., see **Masuya & al.**

Kope, Harry H. & Isabel Leal. A new species of *Lecanicillium* isolated from the white pine weevil, *Pissodes strobi.* 94: 331–340. 2005 (2006).

Leal, Isabel, see **Kope & Leal**

Li, Chunru, see **Huang & al.**

Li, Shigui, see **Huang & al.**

Li, Zengzhi, see **Huang & al.**

Li, Zengzhi, see **Huang & al.**

Liang, Zongqi, see **Han & el.**

Liang, Zongqi, Yanfeng Han, Aiying Liu & Jianzhong Huang. Some entomogenous fungi from Wuyishan and Zhangjiajie nature reserves 2. Three new species of the genus *Hirsutella.* 94: 349–355. 2005 (2006).

Liberato, J. R. & R. W. Barreto. *Streptopodium passiflorae* comb. nov. on *Passiflora rubr*. 94: 89–92 2005 (2006).

Liu, Aiying, see **Liang & al.**

Liu, Pei-Gui, see **Chen & al.**

Maia, Leonor C., see **Silva & al.**

Marchetti, Mauro, see **Iotti & al.**

Masuya, H., J.-J. Kim, M. J. Wingfield, Y. Yamaoka, S. Kaneko, C. Breuil & G.-H. Kim. Discovery and description of a teleomorph for *Leptographium koreanum*. 159–173. 2005 (2006).

Medel, Rosario. A review of the genus *Gyromitra* (Ascomycota, *Pezizales, Discinaceae*) in Mexico. 94: 103–110. 2005 (2006).

Mel'nik, Vadim A., see, **Constantinescu & al.**

Miller, S.L., see **Das** & al.

Mo, Minghe, see **Yang & al.**

Montoya, Leticia & Victor M. Bandala. A new species and a new record of *Lepiota* occurring in the Gulf of Mexico area. 94: 111–125. 2005 (2006).

Orange, Alan, see **Halici & al.**

Özdemır Türk, Ayşen, see **Tufan & al.**

Parmasto, Erast & Robert L. Gilbertson. The genus *Hymenochaete* (Basidiomycota, Hymenomycetes) in the Hawaiian Islands. 94: 189–214. 2005 (2006).

Peintner, Ursula. Book review: *Cortinariales* [Urbonas 2005]. 94: 378–379. 2005 (2006).

Ramaley, Annette W. The connection of *Dothidotthia aspera* (*Botryosphaeriaceae*) to a hyphomycetous anamorphic fungus, *Thyrostoma negundinis*. 94: 127–132. 2005 (2006).

Ríos, Asunción de los. Book review: Fungi of the Antarctic: Evolution under Extreme Conditions [de Hoog, ed. 2005]. 94: 376–377. 2005 (2006).

Rodtong, S., see **Suwannasai & al.**

Şahin, Ahmet, see **Hüseyin & al.**

Selçuk, Faruk, see **Hüseyin & al.**

Sharma, J.R., see **Das** & al.

Sharma, P., see **Das** & al.

Silva, Gladstone A., Leonor C. Maia & Sidney L. Stürmer. A dichotomous key to *Scutellospora* species (*Gigasporaceae*, Glomeromycota) using morphological characters. 94: 293–301. 2005 (2006).

Stürmer, Sidney L., see **Silva & al.**

Sümbül, Hüseyın, see **Tufan & al.**

Suwannasai, N., S. Rodtong, S. Thienhirun & A.J.S. Whalley. New species and phylogenetic relationships of *Hypoxylon* species found in Thailand inferred from the internal transcribed spacer regions of ribosomal DNA sequences. 94: 303–324. 2005 (2006).

Tang, B.-H., T.-Z. Wei & Y.-J. Yao. Type revision of three *Termitomyces* species from India. 94: 93–102. 2005 (2006).

Thienhirun, S., see **Suwannasai & al.**

Torres, Mónica S., James F. White, Jr. & Joseph F. Bischoff. *Cordyceps spegazzinii* sp. nov., a new species of the *C. militaris* group. 94: 253–263. 2005 (2006).

Tufan, Özge, Hüseyın Sümbül & Ayşen Özdemır Türk. The lichen flora of the Termessos National Park in Southwestern Turkey. 94: 43–46. 2005 (2006).

Vanderweyen, Arthur. Book review: Monograph of the genus *Hemileia* (*Uredinales*) [Ritschel 2005]. 94: 383. 2005 (2006).

Vargas-Rodriguez, Yalma Luisa & J. Antonio Vázquez-García. 2005 (2006). *Blumenavia toribiotalpaensis:* a new species of *Clathraceae* from Jalisco, Mexico. 94: 7–14. 2005 (2006).

Vázquez-García, J. Antonio, see **Vargas-Rodriguez & Vázquez–Garcia**

Verkley, Gerard J.M., see, **Constantinescu & al.**

Vernia, C.S., see **Grand & Vernia**

Vieira, Bruno S. & Robert W. Barreto. *Lewia chlamidosporiformans* sp. nov. from *Euphorbia heterophylla*. Mycotaxon 94: 245–248. 2005 (2006).

Villegas, Margarita, see **García-Sandoval & al.**

Wang, Han-Chen & Zhu-Liang Yang. A new species of *Lepiota* (*Agaricaceae,* Basidiomycetes) from China. 94: 51–54. 2005 (2006).

Wang, Yun, see **Chen & al.**

Watling, Roy. Book review: Les Réactifs Mycologiques. Vol. 2. Les Réactifs Microchimiques [Charbonnel 2004]. 94: 377–378. 2005 (2006).

Wei, Jiangchun, see **Wei & Wei**

Wei, T.-Z., see **Tang & al.**

Wei, Xinli, & Jiangchun Wei. Two new species of *Hypogymnia* (*Lecanorales,* Ascomycota) with pruinose lobe tips from China. 94: 155-158. 2005 (2006).

Whalley, A.J.S., see **Suwannasai & al.**

White, Jr., James F., see **Torres & al.**

Wingfield, M. J., see **Masuya & al.**

Yamaoka, Y., see **Masuya & al.**

Yang, Dongshen, Weimin Chen, Ying Huang, Minghe Mo & Keqin Zhang. A new predatory fungus from China. 94: 215-217. 2005 (2006).

Yang, Zhu-Liang, see **Wang & Yang**

Yang, Zhu-Liang, see **Zhang & al.**

Yao, Y.-J., see **Tang & al.**

Yao, Y.-J., see **Yao & Jiang**

Yao., Y. -J., see **Deng & Yao**

Zambonelli, Alessandra, see **Iotti & al.**

Zhang, Keqin, see **Yang & al.**

Zhang, Ping, Zhu-Liang Yang & Zai-Wei Ge. Two new species of *Ramaria* from southwestern China. 94: 235–240. 2005 (2006).

Zhuang, Wen-Ying. Notes on *Otidea* from Xinjiang, China. 94: 365–370. 2005 (2006).

Zyska, Bronislaw. Book review: Checklist of Polish Larger Basidiomycetes [Wojewoda 2005]. 94: 379–380. 2005 (2006).

Reviewers, Volume Ninety-four

The Editors express their appreciation to the following individuals who have, prior to acceptance for publication, reviewed one or more of the papers appearing in this volume.

Teuvo Ahti
T.V. Andrianova
Alan W. Archer
Richard Baird
Victor Bandala
Stephen Bentivenga
M. E. Barr Bigelow
José Luiz Bezerra
Uwe Braun
Paul Bridge
Peter Buchanan
Michael A. Castellano
Shengjun Chang
Andrzej Chlebicki
Vincent Demoulin
Cvetomir M. Denchev
Dennis E. Desjardin
Paul Diedrich
Harry C. Evans
Tobias G. Frøslev
Laura Guzmán Dávalos
Ian R. Hall
Karen Hansen
Maria Havrylenko
Lauraine Hawkins
David L. Hawksworth
Donald E. Hemmes
Kentaro Hosaka
Richard A. Humber
Gintaras Kantvilas
W. Bryce Kendrick
Thomas Kirisits
Richard P. Korf
Shidong Li
Taihui Li
Zong-Qi Liang
Jielin Liu
Xuefeng Liu
Patrick McCarthy
Eric McKenzie
John McNeill
Vincenzo Migliozzi
Joseph Morton
Gregory M. Mueller
Karen K. Nakasone
Tuomo Niemela
Lorelei L. Norvell
David N. Pegler
Ursula Peintner
Antonio Batista Pereira
Shaun R. Pennycook
Ronald H. Petersen
Donald H. Pfister
Scott A. Redhead
Peter Roberts
Jack D. Rogers
Amy Y. Rossman
Mark R.D. Seaward
Keith Seifert
Beatrice Senn-Irlet
Hyeon-Dong Shin
Harrie J.M. Sipman
Brian M. Spooner
James M. Trappe
Adnan Uzunovic
Kálmán Vánky
Larissa Vasilyeva
Jukka Vauras
Else C. Vellinga
Jiangxin Wang
Roy Watling
Zhu L. Yang
Yi-Jian Yao
Rasoul Zare
Wen-Ying Zhuang

MYCOTAXON ONLINE RESOURCES: WWW.MYCOTAXON.COM

Index to fungous and lichen taxa—*Mycotaxon* proudly provides full indices to all authors and taxa that appear in its pages. We continue to print complete indices to authors in each *Mycotaxon* volume. Until 2004 (*Mycotaxon* 1–88), we also printed complete lists of taxa, which are still available for purchase. Because digital files are far more conveniently searched than separate appendices in each print journal, we now post *Mycotaxon* taxonomic indices only online. Taxonomic indices are now current up through Volume 91, but Volumes 92–94 are scheduled for posting later in 2006. To read or print *Index to fungous and lichen tax*a for **Volume 91**, download our posted PDF file by clicking first on INDICES (in the list at the top of any webpage under the *Mycotaxon* header) and then TAXON INDEX, VOL 91.

Cumulative indices—Readers can now swiftly and easily locate taxonomic and author names in the cumulative indices to authors and taxa from earlier *Mycotaxon* volumes. Cumulative author indices available are VOLUMES 61–93 on the *MYCOTAXON* website and Volumes 1–20 on the CYBERLIBER website. Cumulative taxonomic indices now available for downloading include TAXON INDEX, VOLUMES 61–70; TAXON INDEX, VOLUMES 71–80; and TAXON INDEX, VOLUMES 81–90. Taxonomic entries for Volumes 91–94 will eventually be integrated into TAXON INDEX, VOLUMES 91–FF.

Distributional checklists—*Mycotaxon* posts downloadable PDF files or URL links first published in *Mycotaxon* by authors who amend or update species identifications, nomenclatural revisions, and range & substrate extensions as new data become available. To find the checklist page, first click ONLINE RESOURCES (under the *MYCOTAXON* header) and then REGIONAL CHECK LISTS. Users may also find checklist PDFs and URLs by clicking on links in the summary abstracts. (Abstracts are found following the PUBLICATIONS -> VOLUME LISTING –> VOLUME NUMBER -> TITLE pathway.)

Volumes 1–27 now online—*Mycotaxon* happily announces that *cost-free access to papers in back volumes* is well underway. Early volumes may be accessed and individual pages downloaded by following the CYBERLIBER –> BOOKS & JOURNALS –> *MYCOTAXON* pathway from the *MYCOTAXON* homepage.

Information for authors and reviewers—The INSTRUCTIONS TO AUTHORS page is the best way to find the most up-to-date information needed for *Mycotaxon* manuscript submission. Downloadable files include the 19-page detailed INSTRUCTIONS TO AUTHORS PDF and the following document files: REVIEWER GUIDELINES (with peer review checklist for expert reviewers), official *MYCOTAXON* SUBMISSION FORM, and our pre-formatted *MYCOTAXON* WORD SHELL.

Search—The **search!** engine option in the top right corner of every *Mycotaxon* webpage is yet another way to search for authors, taxa, and other online resources. Currently it only indexes taxa cited in titles, abstracts, or keywords of published papers. The search button also finds all nomenclatural novelties.

Errata

Volume 3

The new generic name *Burenia* is misspelled on pp. 1, 9-12, 46-47, and 570. It is an orthographic error, correctable under the International Code of Botanical Nomenclature. The name honors G. von Büren, misspelled Buren on pg. 11, but spelled correctly on pg. 48. All the Latin names cited should thus be corrected to *Buerenia.* We are advised that the correct spelling will appear in the 10th edition of *Dictionary of the Fungi* (CMI).

Volume 91

pg. 28, ln. 11	for: *Thecispora*	read: *Trechispora*
pg. 36, f. 5 caption	for: *Coltricella*	read: *Coltriciella*
pg. 129, ln. 4	for: *P. redivivus*	read: *Panagrellus redivivus*
pg. 130, ln. 11	for: *tenius*	read: *tenuis*
pg. 137, abstr. ln. 8	for: *fomitiporoides*	read: *fomitoporoides*
pg. 144, ln. 8	for: *fomitiporoides*	read: *fomitoporoides*
pg. 154, ln. 17	for: *fomitiporoides*	read: *fomitoporoides*
pg. 224, ln. 18	for: *segetum*	read: *segetum* ∂
pg. 224, ln. 18	for: *carbo*	read: *carbo* ∂
pg. 224, ln. 19	for: Wallr.	read: Wallr. ß
pg. 226, par. 3, ln. 2	for: *versatilis*	read: *versatile*
pg. 250, ln. 5	for: *tanganykeanum*	read: *tanganyikeanum*
pg. 362, ln.15	for: *griseiviridis*	read: *griseoviridis*
pg. 363, ln. 2	for: *griseiviridis*	read: *griseoviridis*
pg. 526, ln, 12	delete *Pertusaria hueangensis* Jariangprasert, p. 283	
pg. 526, after ln. 21	add: PHAEOTREMATACEAE Popoff ex Piatek, p. 181	
pg. 526, ln. 21	for: *parvicarpum* ... 352	read: *parvicarpum* ... 351
pg. 527, ln. 2	for: *namaqualandensis*	read: *namaqualandus*

Volume 93

p. 379, line 4	for: *aurelio@castillo@uah.es*	read: *aurelio.castillo.uah.es*

Instructions for MYCOTAXON Authors

(Updated December 2005)

MYCOTAXON is an international mycological journal devoted to research on the taxonomy and nomenclature of fungi. Publication is open to everyone. Authors are responsible for obtaining *peer reviews* from experts in the field prior to presubmission of their manuscripts to the *Nomenclature* and *French Language Editors*. The *Editor-in-Chief* reviews all expert peer reviews, presubmission recommendations, and author submissions and prepares all files and hard copy for printing by *Sheridan Press,* which also handles reprints. Subscriptions, author invoices, and payments are handled by the *Business Manager.* MYCOTAXON is indexed by the *Index Editor* and the *Webmaster* is responsible for maintaining the journal website on *www.mycotaxon.com.*

Review Editors

Dr. Lorelei L. Norvell, MYCOTAXON Editor-in-Chief
<editor@mycotaxon.com>
6720 NW Skyline Boulevard
Portland OR 97229-1309 USA

Dr. Shaun R. Pennycook
MYCOTAXON Nomenclature Editor
<PennycookS@LandcareResearch.co.nz>

Prof. Dr. Grégoire L. Hennebert
MYCOTAXON French Language Editor
32 Rue de l'Élevage
B-1340 Ottignes-LLN, BELGIUM

Authors: Include 'MYCOTAXON + author name + date' *or* 'MYCOTAXON accession number' in all subject headers. Add *editor@mycotaxon.com, llnorvell@pnw-ms.com, info@mycotaxon.com,* and *PennycookS@LandcareResearch.co.nz* to your Email-friendly address lists.

Submissions are usually acknowledged within two weeks of receipt, although acknowledgments may slow during field season or at press times. Manuscripts go first to the Nomenclature Editor, who assigns permanent accession numbers. Completed manuscripts and supporting materials go to the Editor-in-Chief, who reviews final submissions in the order received, returning files for revision as needed before preparing the final papers for the press. Author listings, page numbers, and proof PDFs sent for final author approval must be acknowledged within three working days.

Only errors introduced by PDF conversion are corrected free of charge. Conversion errors include omitted text, altered symbols, and other anomalies introduced by the Editor-in-Chief, but not minor shifts in text flow caused by graphics insertion. MYCOTAXON reserves the right to reject papers of questionable taxonomic merit or those that do not meet other listed criteria.

Overview

1. What is suitable for publication in MYCOTAXON? *p.402*
2. Submission procedure *402*
3. Preparing text *403*
4. Preparing illustrations *407*
5. Peer review *409*
6. Nomenclatural & French Language review *409*
7. Final submission *409*
8. Final author checklist *410*
9. Corrections Policy *411*
10. Obtaining reprints *411*

1. What is suitable for publication in Mycotaxon?

Mycotaxon is restricted to papers on the **taxonomy and nomenclature of fungi.** We intend this broadly to include monographic works, reviews of taxonomic groups and/or taxonomic criteria, arguments dealing with specific nomenclatural problems, proceedings of symposia on taxonomic or nomenclatural matters, and well-documented floras. Papers that deal with other mycological disciplines (cytology, ecology, genetics, phylogenetics, physiology, etc.) should be submitted to another journal unless their *primary focus* is taxonomic. Prospective authors are encouraged to send a draft to the Editor-in-Chief for consultation regarding suitability of their manuscripts for publication in the journal.

Articles may be of any length. Most authors pay no page charges, although sole/senior authors who exceed 100 pages per year may be charged $18 per excess page. Authors are granted one halftone plate for every ten (10) pages of text (11-20 text pages qualify for 2 halftones, 21-30 pages for 3 halftones, etc.). Fees are $40 for each excess halftone plate. Black & white line drawings, phylotrees, graphs, and tables are not considered halftones and count as text in assessing page charges. There are also fees for digitizing original artwork, converting non-standard graphics files to TIF or EPS format, and correcting author errors in PDF proofs.

About regional (distributional) checklists

Until 2003, Mycotaxon published annotated regional checklists of fungi, which often served as first fungal records from under-explored areas. Now, web-based documents offer a searchable interface and permit rapid integration of new information. Thus, Mycotaxon no longer publishes regional complete fungal inventories in the print journal, instead inviting authors to publish 1- to 4-page summaries of longer checklists posted on the Internet. Both URLs to checklists posted on authors' own websites and downloadable PDF checklists are posted on <**www.mycotaxon.com/weblists/**>. There is a one-time $25 posting fee for uploading a PDFed distributional paper onto the Mycotaxon weblist page.

Summaries must adhere to the same editorial requirements as regular manuscripts. Both original checklists and summaries undergo peer and nomenclatural review before final submission to the Editor-in-Chief. Checklist authors are *strongly* encouraged to cite references used to identify the taxa in their research and must include the URL to their checklist in the abstract. Authors wishing to post on Mycotaxon's webpage should contact the Business Manager <**info@mycotaxon.com**>, who will upload their PDF checklist after final editorial approval. All PDFs, which may be updated for a $10 revision fee, must display original submission and revision dates on the first page.

2. Mycotaxon submission procedure

Authors are encouraged to download the **more detailed instruction** PDF (containing full clone preparation instructions + sample manuscript), and Reviewer Guidelines + Checklist, Mycotaxon Word® shell, and submission form document files from the Instructions to Authors webpage on <www mycotaxon.com> before preparing a manuscript for Mycotaxon. (All files will also be E-mailed by the Editor-in-Chief on request.)

Authors who wish to prepare their own press-quality PDFs using a desk-top publishing application must pre-flight their files through Sheridan Press before final submission and be able to insert masthead and page numbers exactly as requested at press-time. Those intending to submit text for scanning and digital conversion should first contact the Editor-in-Chief for special instructions.

Please be aware that editors open **only** E-mails that have "Mycotaxon" followed by author name and reference number or title word in the subject headers. Authors should add <editor@mycotaxon.com>, <llnorvell@pnw-ms.com>, <PennycookS@LandcareResearch.co.nz>, and <info@mycotaxon> to their list of permitted incoming addresses. Authors who mail hard copy are reminded to package all materials securely, enclose address information within the packet, and to use stiff boards, bubble wrap & plastic cases to protect artwork/CDs.

Peer Review (Step 1)

Authors send formatted manuscript **text** & **graphics** files with the MYCOTAXON instruction PDF and reviewer guidelines to *two experts* for presubmission peer review. English grammar, nomenclature, and author citations must be checked by at least one reviewer.

Peer reviewers (i) return edited manuscripts to authors and (ii) E-mail forms & review summaries to authors & MYCOTAXON Editor-in-Chief immediately after the first review.

Nomenclature & Presubmission Review (Step 2)

Authors send revised & formatted **master text clone** (*no* graphics) and peer reviewer E-mail addresses to the *Nomenclature Editor* and (when appropriate) *French Language Editor*.

The *Nomenclature* & *French Language* Editors return annotated clones with a list of needed corrections to the authors, peer-reviewers, and Editor-in-Chief.

Authors correct manuscript (consulting peer reviewers when necessary).

Final submission (Step 3)—Authors E-mail or post the Editor-in-Chief

Manuscript PDF/MSWORD preview file *or* print copy with fully formatted, legends, tables & footnotes with graphics either embedded or placement indicated;

Master & body text clones (and table/legend clones when tables/figures are included);

Graphics as original hard copy or digital JPG/TIF/EPS file. All images must set in grayscale mode. Photo halftones must have a minimum of 300 dpi resolution; black & white line art must have 900-1200 dpi resolution. (All resolutions are scaled for a 4.33" or 11 cm print area width.) (Contact the Editor-in-Chief to determine fees for scanning hard-copy original artwork and converting embedded megafiles or MSWORD pictures to required JPG/TIF/EPS formats); and

MYCOTAXON submission form and short cover letter.

Editorial review and press-preparation (Step 4)

The *Editor-in-Chief* (i) acknowledges submission (usually within 2 weeks) and (ii) after editorial review *either* E-mails approval and requests author statement that clones are accurate *or* returns marked clones to the author for revision.

Authors return revised files and/or a paragraph stating that all text is error-free and that the Editor-in-Chief may adjust graphics, legends, and tables as needed during press preparation.

The *Editor-in-Chief* merges all author files in InDesign to prepare a press-quality PDF file. The PDF proof is sent to the authors for final inspection before going to press. All fees should be sent to the *Business Manager* at this time.

3. Preparing text and text clones

Authors must prepare concise, well-formatted, error-free copy that accurately conveys the author's ideas with an economy of space. Articles may be written in either English or French. Authors submitting copy in English should follow one uniform spelling and grammatical convention (American or British) throughout the manuscript.

Digital submission is encouraged, with MSWord® text files and Adobe Photoshop® graphics files preferred. Sheridan Press, which prints MYCOTAXON, provides a PDF booklet <http://www.sheridanpress.com/whitepapers.htm> that explains how to prepare illustrations for publication. The Press also permits authors to submit up to 3 digital TIF files to "Digital Expert" <http://dx.sheridan.com/> for free diagnosis of potential problems prior to submission. Authors wishing to prepare their own press PDF files must contact the Editor-in-Chief for special instructions and pre-flight their PDF with Sheridan Press *before* final submission.

Hard-copy submission is still accepted, but authors are asked to ask the Editor-in-Chief for additional guidance before submission. Mycotaxon now charges a $5 per page/illustration scanning fee to digitize hard copy and is unable to scan artwork larger than 23 × 31cm (9 × 12").

Clones: What are they and how are they used?

Mycotaxon asks authors to submit text clones for nomenclatural and final editorial reviews. These 'clones' contain all formatted text that will appear in the published paper, but no illustrations, which should be submitted separately. The four text clone types are *master* (for review & final submission) and *body, legend,* and *table* (for final submission). Authors submitting preflighted PDFs approved by Sheridan Press for publication need not submit clones.

The **master** text clone contains ONLY text (*no* graphics) used during peer and nomenclatural review where Mycotaxon masthead mockup, tables, legends, footnotes, breaks, and empty lines indicating graphics placement are all present. The **body** text clone is the master clone with masthead, empty paragraph lines, tables, footnotes, legends, and line, section & page breaks *removed.* The **legend** text clone contains all footnotes & legends with explanatory text for placing each footnote and illustration. The **table** text clone contains all formatted title+table+footnote text. (Two table text clones are submitted when tables have differing (i.e., landscape vs. portrait) orientations.) **Text clones must be correct!** The complete manuscript is used *only* as a placement guide for tables and figures. The clones are combined with illustrations to generate the final PDF press file.

> Pages 7–8 in the 'Mycotaxon Instructions to Authors' PDF (which can be downloaded for free from www-mycotaxon.com website) explain in detail how to prepare clones. Authors are also encouraged to write the *Editor-in-Chief* for additional assistance if needed.

Text must conform to the following specifications

Print area size is 11 × 17.5 cm (4.33 × 6.89"). The Mycotaxon Word shell has been formatted for a US letter (A3) paper size with 5.25 cm top/bottom & 5.3 cm side margins. It also contains a mock-up of the Mycotaxon logo and header for the first page. Those using other word-processing applications should follow the above guidelines, allowing 2.5 cm (1") for the title page masthead on the first page.

Fonts & Paragraph formatting—Authors are asked to use two basic font families: serif TIMES or TIMES NEW ROMAN (TNR)—and sans serif ARIAL or HELVETICA (HELV). Characters not displayed on keyboards (α, β, μ, ×) should be selected from the SYMBOLS menu if not available in the regular fonts. Courier is used only when comparative columnar arrangement is essential (e.g., for DNA sequences). (**All** other fonts require editorial permission.) Lines *must* be single-spaced, and text formatted with 1 1/2 spaces between lines is *not* acceptable. Authors should use *paragraph format menu* options rather than hit the return key twice to separate paragraphs or stand-alone subheadings or use the tab key to indent paragraph first lines by 0.5 cm.

> Note: Standard elements of an article are the title, author address information, abstract, key words, main text, figure legends, acknowledgments, and references. The Mycotaxon instruction PDF offers a properly formatted sample manuscript and includes other helpful manuscript preparation suggestions.

Required formats

- **Title**—*Font:* Arial, 11-pt, Bold, **sentence** case (*never* upper or title); *Paragraph:* no full stop (dot) at the end (unless ending with an abbreviation), no indent, center aligned.

Special: Set Latin scientific names in ***bold italic.*** Titles should not exceed three lines. Author citations are not acceptable unless authors demonstrate why an authority is necessary to the title. Abbreviate genus names after the first use, but otherwise avoid abbreviations. Use arabic (not roman) numerals. *Example:*

Studies in *Agaricales* 3:
Phaeocollybia phaeogaleroides* and *P. rifflipes,
new western North American species

- **Author names**—*Font:* Times, 10-pt, Roman (regular), 'SMALL CAPS'; *Paragraph:* no indent, center aligned.

Special: Given names and initials always stand before surnames. Separate authors with commas, but use '&' before the last author's name.

- **Address information**—*Font:* Times, 9-pt, Italic; *Paragraph:* no indent, center aligned, no periods at line ends; *Placement:* E-mail address (required*) on top line; Institution/Street on middle line; City, Code, Country on bottom line.

Special: *Junior author E-mail addresses recommended, but optional. Do not end lines with commas or periods (except for abbreviations).

- **Abstract**—*Font:* Times, 8-pt; set '**Abstract**' and '**Key words**' in bold and precede em-dash and remaining paragraph/list formatted in regular or italic as needed. *Paragraphs:* 1 cm right & left margins, no indent, fully justified.

Abstract: Abstracts briefly summarize the content & conclusions and list all new taxa (but not authorities unless differentiating homonyms). English abstracts are required; 1–2 abstracts in other languages are permitted for longer articles. Abstracts should not exceed 15 printed lines.

Key words: Up to five key words or phrases are permitted. Do not repeat terms already used in the title or abstract. Separate list items with commas. Capitalize only proper nouns.

- **Subheadings: Primary (stand-alone)**—*Font:* Arial 10-pt, Bold/Bold Italic; *Paragraph:* no indent, center aligned. **Secondary (stand-alone)**—*Font:* Times 10-pt, Bold/Bold Italic; *Paragraph:* no indent, left aligned. Arial 10-pt regular (roman) and Arial 9-pt, Bold/Bold Italic are optional secondary subheadings.

Taxonomic (special)—*Font:* Times; *Size & Style:* **Latin name**—10-pt Bold Italic, **Author citation**—9-pt regular; '**nom. nov.**' & '**Fig. #**'—9-pt bold. *Paragraph:* hanging indent, left aligned; set right tab to 11 cm and use tab to justify Fig. # at right.

- **Basic text**—*Font:* Times 9-pt; *Paragraphs:* fully justified. Select 6-pt gaps or 0.5 cm first line indents to separate paragraphs (*not* both).

Introduce your subject briefly by citing significant background literature. Provide necessary abbreviations and other data in a brief Materials & Methods section (8-pt font permitted here). Document your own observations concisely, stressing new discoveries. Minimize confusion between generic epithets beginning with the same initial letter by abbreviating one using the first **two** letters of the generic name. Italics are required for Latin scientific names of taxa up through and including order or reserved for emphasis; common Latin abbreviations (e.g., i.e., inter al., etc.) and references are **not to** be italicized. List author citations *once* only: in the taxonomic heading where a new taxon is proposed *or* where first mentioned in the text. Authors should list previously misidentified taxa as 'misapplied' *not* 'sensu + author + reference.'

Required references: Consult the current INTERNATIONAL CODE OF BOTANICAL NOMENCLATURE <http://www.bgbm.fu-berlin.de/iapt/nomenclature/…> when describing new taxa or proposing new combinations. Author citations *must* follow either the updated Kirk & Ansell's AUTHORS OF

FUNGAL NAMES <http://www.indexfungorum.org/Names/Names.asp> or INTERNATIONAL PLANT NAMES INDEX <http://www.ipni.org/ipni/query_author.html>. Consult INDEX HERBARIORUM <http://www.nybg.org/bsci/ih/searchih.html> for herbarium/collection acronyms.

Authors describing new taxa *must* cite relevant acronyms and numbers to facilitate retrieval by readers *and* deposit new names/data in **MycoBank** <http://www.mycobank.org>, type specimens in an official public **herbarium**, ex-type strains in a public **culture collection**, and/or sequence data in **GenBank** <http://www.ncbi.nih.gov/Genbank/submit.html>.

- **Latin diagnoses, nomenclators, and 'Specimens examined'**—*Font:* Times, 8-pt; *Paragraph:* margins indented ~1cm, fully justified with first line flush. *Special:* Italicize all Latin text in diagnoses but only Latin scientific names in nomenclators & 'Specimens examined.'

- **Acknowledgments**—*Font:* Times, 8-pt. *Paragraph:* no indent, left aligned, fully justified. *Special:* Acknowledge or thank pre-publication peer reviewers here.

- **Literature cited**—*Font:* Times, 8-pt; *Paragraph:* Hanging indent, no line spaces between entries. **Required:** All references must be cited in the main text. Follow a consistent citation style throughout. Never use more than one space between entries. *Author names:* substitute initials (no periods after initials) for given names, order surnames before initials and do not separate with commas, separate author entries only by commas and never by "&" or "and", and place a period (dot) after the last author entry. Standardize journal abbreviations.

Examples (consult also MYCOTAXON sample manuscript in Instructions PDF):

Kirk PM, Cannon PF, David JC, Stalpers JA. 2001. Ainsworth & Bisby's dictionary of the fungi. CAB International, Surrey UK.

Senior AA, Junior BB. 1997. Title with *Latin name* here. MYCOTAXON 56: 254-272.

Useful references: See BOTANICO-PERIODICO-HUNTIANUM (BPH: Lawrence & al. 1968), BOTANICO-PERIODICO-HUNTIANUM/SUPPLEMENTUM (BPH/S: Bridson 1991), TAXONOMIC LITERATURE, (TL: Stafleu & Cowan 1976-1988), and SUPPLEMENTS TO TAXONOMIC LITERATURE (TL2: Stafleu & Mennenga 1992-1995) for recommended abbreviations.

Pay particular attention to the following

Hyperlinks—*Disable* hyperlinks in all clones. Clones with active hyperlinks will be returned to the author for correction. Be aware that text imported from PDFs or websites frequently retains the original hyperlinks or appear in foreign [unrecognizable] fonts during the press conversion process. When in doubt, authors should retype the text themselves.

Italics & underlines—Reserve *italics* **only** for Latin taxonomic names or emphasis. Names of all taxa from subspecific to ordinal levels *must* be italicized; using italics for higher-level names is at the author's discretion. Do **not** italicize common 'Latin' abbreviations (e.g., et al., etc., inter al.) or reference titles. **Never** underline text intended for publication.

Symbols—Generate diacritical marks and symbols (e.g., ä, ñ, μ, ×) by using special keystrokes (e.g., "option+m" for μ) or by inserting from the 'symbols' menu. When possible, select symbols from the corresponding text font; in Word insert 'μ' via the Insert > Symbol > Times pathway. Some symbols (e.g., '×') exist only in the 'Symbols' *font.* Authors should list symbols inserted from the Symbols (instead of the Times/TNR menu) on the submission form.

Punctuation—Place commas, periods, and other punctuation in the same font style as the word directly preceding them. For instance, commas stand in italics after words in *italics,* in bold after words in **bold,** and in bold italic after words in ***bold italic.*** However, the closing mark of paired marks such as parentheses, square brackets, quotation marks, single quotation marks, and long dashes exhibits the same font style as its companion open mark, *even* when preceded by a word in a different style. With the exception of the **sanctioning colon** (e.g., Fr. : Fr.), **no** space stands between a punctuation mark and the preceding text. Likewise, no space stands between an initial paired mark (e.g., open parenthesis) and the first word. In computer-formatted manuscripts, only **one** space follows a full stop at the end of a sentence.

The *hyphen*, *en-dash*, and *em-dash* have different uses—The *hyphen* separates word elements and breaks at line end. Do **not** introduce hyphens to long words in text clones as they may appear in midline after PDF conversion. The *en-dash* (keyed on a 'Mac' using 'option+dash') is longer, and does not break at line end. Use an en-dash with spaces for 'minus' in mathematical notation and without spaces in range expressions: the en-dash in '8–10' keeps the '8' and '10' together on one line. The *em-dash* ('shift+option+dash' on a Mac) is the longest and—generally—is reserved for emphatic clauses that are otherwise enclosed in parentheses. No spaces are used.

Spelling and grammatical errors—*All* co-authors should proofread text for spelling, typographic, and grammatical errors; grammar and spell checkers can also be helpful. The two most common spelling errors are still (i) misspelling authors' own names & addresses (!) and (ii) spelling names of taxa one way in the text and another way in figure captions.

Non-native English speakers *must* ask someone fluent in English or French to proofread the paper before *each* review (peer and nomenclatural) and again before final submission. Additional attention should be given to Latin diagnoses, which should be **brief**. Diagnoses that list only the key characters separating a proposed new taxon from closely related ones generally contain fewer errors than more complicated descriptions. Save details for the technical description.

4. Preparing illustrations

> ***Color option:*** Those wishing to publish color illustrations should correspond with Sheridan Press before final submission. Current press charges are $475 per color page.
> ***Returns:*** MYCOTAXON will return original illustrations to authors who include a self-addressed envelope that is *stamped* or submitted with sufficient *International Postal Reply Coupons,* with their final submission.

Size & placement

All articles published in MYCOTAXON begin on an odd-numbered (right-hand) page. It is preferable to place a figure on a right-hand page facing the text first referring to the illustration. Group figures to facilitate species comparisons and keep page numbers to a minimum. Do not border or edge photographs and drawings with a line. Illustrations are best formatted at publication size. Plan to place figure legends below figures (preferred) or at the bottom of pages *facing* illustrations.

Line drawings

Authors may submit originals, digital images prepared from scanned originals, or high quality reduced photocopies of original artwork. Authors with access to computer scanners and photographic software are encouraged to digitize original drawings themselves.

Digital files— Digital files that incorporate fonts or vector information should be submitted as 1200dpi TIF (in bitmap mode) or 900dpi EPS files. Simple line drawings can be scanned using software such as ADOBE PHOTOSHOP®, but more sophisticated illustration software (e.g., ADOBE ILLUSTRATOR®) should be used to convert phylotrees and other drawings containing vector information to EPS format. Image adjustments — e.g., insertion of scales, numbers, and arrows — should be handled in such applications. Remember that digital figures should *not* include legends, which belong in the legend text clone.

Hard copy— Authors who submit digital text with hard copy artwork may include photocopies within a manuscript printout to indicate figure placement; however, they should also submit line drawings separately *without* legends. Authors who submit exclusively hard copy materials should position line drawings onto existing text pages *with* captions printed directly below or to the side of each drawing. With care, authors generally scale down oversized drawings using the reduction feature on photocopiers without decreasing image quality and assemble an attractively arranged plate from the cut & paste illustrations and captions.

Photographic 'halftones'

Mycotaxon prints only grayscale ("black & white") photographs, but authors who submit film-based (not digital) prints may submit color photos for conversion to grayscale if necessary. Both grayscale and color photographs have a "continuous tone" requiring transformation into a separate *halftone image* before they can be printed for publication. (A halftone image consists of many variously sized and spaced dots, which are easily seen using a hand lens.).

Digital grayscale halftones—Photographic software such as Adobe Photoshop® allows preparation of compressed jpg files (useful for peer review) and tif files (non-compressed files used in journal publications). Tif or jpg halftones **must** be set in *grayscale* mode with a minimum resolution of *300-600 dpi* (dots per inch) matching the target width, generally 4.33" (11 cm). Authors who group photographs into **plates** should ensure that the contrast among individual photographs is not too great; individual photographs within plates may be set flush or separated by a 1-2mm white or black band. Authors who prepare their own pdfs for printing may insert both line and photographic art using publishing software (e.g. InDesign®).

Negative-based photographs—To prepare negative-based photographic plates, crop individual photographs so that edges touch and the plate is exactly 4.33" (11 cm) page wide. Mount photographs on separate sheets of heavy white paper or matte board. Apply annotations (arrows, numbers, scales, etc.) and band strips after the photographs are positioned and be certain to allow space around the photograph for editorial markings. **NOTE:** Authors must **not** submit halftone prints prepared from digital files as hard copy intended for publication; they should submit instead grayscale computer files formatted as described above.

Labeling

Title all graphic files (jpg, tif, eps) with the *first* author's name and *figure* number (e.g., *name_fig#.tif*). Label all original artwork hard copy with the first author's name and figure number on the **reverse** side.

Final note about digital images

The Editor-in-Chief is able to convert a good many different file formats quickly to tif or eps files in Adobe Photoshop®. Therefore, there are no fees for converting photoshop, bmp, jpg, pcx, pict, pixar, png, scitex CT, or targa files, provided they open in Photoshop in proper grayscale/bitmap mode and with the required resolution. A $5 fee is charged for processing each MSWord embedded mega- or picture file through Illustrator® before press.

The Editor-in-Chief cannot improve the quality of a bad image. Authors should submit as clear and clean an image as possible but must remember that, in science, reality supersedes beauty. Do **not** 'over-photoshop' or otherwise distort images.

Authors who do use artwork to enhance certain features within a photograph must explain what has been added in the accompanying figure legend.

5. Peer review

Mycotaxon is unusual among scientific journals in that authors are expected to obtain their own peer reviews *before* submission by contacting **two** scientists in their field but *outside* the senior author's home institution. When authors are uncertain whom to approach for manuscript review, they may send their title and abstracts to the Editor-in-Chief for the names of suitable reviewers. Although both mycotaxon Nomenclature Editor and Editor-in-Chief will review each manuscript and may solicit outside reviews when necessary, English grammar, nomenclature, and author citations must have first been thoroughly checked by at least one of the two peers. Authors whose first language is not English are urged to contact a native English-speaking expert to serve as one peer-reviewer.

The corresponding author sends each reviewer the Reviewer Guidelines & Checklist document file with one of the following: a master text clone with individual jpg files, a msword manuscript with embedded figures, or a printed manuscript with photocopied figures. (Guidelines and checklist can be downloaded from <http//:www.mycotaxon.com> or E-mailed by the Editor-in-Chief). After review, each expert returns annotated text to the corresponding author and sends the formal checklist and brief comments to the corresponding author and the Editor-in-Chief.

Authors should revise manuscripts following reviewer suggestions and must explain during final submission which recommendations were not followed and why. They may also ask experts for additional guidance during the post-nomenclatural review revision and may add reviewers who make major improvements as co-authors. The Editor-in-Chief acknowledges all peer reviewers in the closing pages of each volume, but authors should also thank peer reviewers in their own acknowledgements.

6. Nomenclatural and French Language Review

After peer review, all authors must E-mail their master text clone file to the Mycotaxon *Nomenclature Editor*, who reviews text for adherence to the International Code of Botanical Nomenclature and standardization of author citations. Manuscripts written in French should be sent to the *French Language Editor* at the same time. These editors determine whether a manuscript is ready for final submission and return annotated clones with a list of needed corrections to the authors, peer-reviewers, and Editor-in-Chief. Graphics files are to be sent **only** by editorial request. Be certain to write 'Mycotaxon review: [Senior author surname] [genus/order name] [date]" in E-mail subject headers.

7. Mycotaxon final submission

Final submission materials should be sent to the Editor-in-Chief. Materials may be **E-mailed** or sent by **registered airmail** (*signature required*). (Ask permission before E-mailing messages & attachments exceeding 10 mgb.) Authors who intend to submit print-ready manuscripts for scanning should contact the Editor-in-Chief before preparing their final submission. The following items must be received before final manuscript review is begun by the Editor-in-Chief:

Two expert peer reviews—*[sent by reviewers to the Editor-in-Chief]*

Presubmission reviews from the Nomenclature Editor (& **French Language Editor**, when the submission is in French)—*[sent by Editors to Editor-in-Chief]*

Manuscript Text—[**a**] One PDF/document file *OR* a manuscript printed to show approximate figure placement. Print hard copy on only one side; pages may be stapled and have author + page number headers *unless* intended for scanning. [**b**] **Master text clone** and **body, legend, & text clone** document files as needed. (Consult 'Text & text clones' above and the posted Mycotaxon author instruction pdf for more information.)

Illustrations—***Digital:*** E-mail TIF or EPS files (preferred) or airmail on CDs. Other graphic formats are also accepted, but fees are accessed for time-consuming file conversions. ***Hard copy:*** See instructions above. There is a $5 per page or figure to be scanned.

Brief cover letter accompanied by MYCOTAXON submission form—The submission form can be downloaded from the Mycotaxon webite.)

Consult the **Author's Checklist** below before sending the final submission. Remember to prepare back-ups to safeguard against loss or damage to files in transit.

8. Author Checklist

All authors are responsible for providing error-free, properly formatted text and will benefit by downloading the MYCOTAXON Word shell and the expanded Instructions for Authors PDF files from <http//:www.mycotaxon.com/instructions.html >. Those submitting press-PDFS and photo-ready hard copy are also both *designer* and *printer* of their paper and acknowledge that the Editor **cannot** change submitted text. Authors who do not understand instructions are urged to ask their peer reviewers or the Editor-in-Chief for help!

- **Text format (very important)**

— Fonts and paragraphs all conform to *MYCOTAXON* requirements (see above).

— The backs of camera-ready manuscript pages are numbered and labeled with a soft pencil.

— The ***body clone*** file contains no empty paragraph lines, initial tab settings, line breaks (main title excepted), or graphics files. All footnotes, legends, and tables are removed.

— The ***legend clone*** file contains only footnotes & figure legends, each accompanied by instructions noting on which page to place the item in the final manuscript.

— All tables are placed in one ***table clone***. If both 'landscape' and 'portrait' tables are present, they may be placed in two clones according to orientation.

— The **title** is in Arial 11-pt **bold** (Latin names in ***bold italics***), sentence-case, and does not end in a full stop (abbreviations excepted). Taxonomic authorities are not included.

— Manuscript author **names** are centered & in Times, 10-pt roman (regular), 'SMALL CAPS.'

— **Addresses** are centered, in Times, 9-pt *italic,* with **E-mails** at top with hyperlinks disabled, **institutional** information in the middle, and **Street -> Country** information at bottom.

— The **Abstract** and **Key words** are centered with margins indented by 1-cm and in Times, 8-pt font. '**Abstract**' and '**Key words**' are in bold and other text is in regular except for italicized *Latin names.* The key words list contains only five terms separated by commas, does not repeat words in title or abstract, and does not end with a full stop.

— **ALL** text is single-spaced. Leading is 10-pt for paragraphs containing 8-pt font.

— **Latin scientific names** for all taxa from subspecies through order are *italicized.*

— **Author citations** conform to INSI or Kirk & Ansell, occur only once for each taxon, and have been checked by the Nomenclature Editor and at least one peer reviewer.

— No commas stand between author names and (first) dates in **text references.**

— **Literature cited** references follow a consistent order, conform to MYCOTAXON guidelines, have hanging indents, and are in 8-pt Time/TNR.

- **Illustrations**

— **Graphics** are ≤ 11 × 17.5 cm ***or*** authors have editorial permission to send oversized plates.

— **Photographs or composite plates** fill the entire width of the page (except where text or legend takes part of the width)

— **Digital halftones** are in TIF format, grayscale mode, and 300-600 dpi per 4.33" width.

— **Figures** submitted for scanning are mounted separately from the text on heavy stock and are clearly numbered & labeled with author name & brief title on the reverse side.
— **Legends** are separate from artwork. They do not contain taxonomic author citations.
— The corresponding author has **dated backup copies** of all materials submitted.

- **Final submission items**

— *Peer review checklists* & comments have been sent by two expert reviewers to Mycotaxon.
— *Master text clone* has been reviewed by the Nomenclature Editor.
— *Master text clone* written in French has been reviewed by the French Language Editor.
— Final *master text and body clones* (with *legend & table clones* as needed)
— *Illustrations* digital files of proper resolution or hard copy
— PDF / MSWord file or printed *manuscript* showing figure placement
— Brief cover letter and completed Mycotaxon *submission form*

9. Mycotaxon corrections policy

Mycotaxon has always expected authors to submit manuscripts that are free of errors and ready to publish when they arrive on the editorial desk. *All* coauthors are assumed to have viewed and approved text prior to submission. Immediately after final editorial approval, all must again double-check all files to return error-free clones to the Editor-in-Chief for processing.

The Editor-in-Chief processes clone and graphics files using Adobe InDesign®. Text may shift during this process, but InDesign generally distributes text evenly and displays few unsightly gaps in justified lines. Authors receive a pre-publication PDF for final approval and so will have a chance to correct errors introduced by PDF conversion.

Correction of author errors after PDF conversion is costly and time-consuming: Mycotaxon now charges a *minimum* of **$10** *per error* to correct mistakes present in approved clones and **$60** per error to correct mistakes discovered after the files have been sent to press. There is **no** charge for correction of author errors when authors send corrected clones to the Editor-in-Chief before the PDF-conversion process is begun.

Mycotaxon corrects errors resulting from computer or editorial error free of charge. [Errors present in approved clones are considered author and *not* conversion errors.] Mycotaxon also lists, free of charge, corrections of all published errors in the Errata.

10. Ordering reprints or downloadable PDFs

Mycotaxon sends all authors a print-quality PDF file before publication and one copy of the printed article to the corresponding author immediately after publication; a local printer can make high quality reprints from either PDF or print. A PDF explaining how to order 100+ reprints or arrange for Sheridan Press to host the author's PDF file on its website can be downloaded from <http://www.mycotaxon.com/authors/reprints.html>.

Still have questions?

Contact the *Editor-in-Chief* at **editor@Mycotaxon.com**
OR
Download the 'Mycotaxon Instructions' to Authors PDF and
check the FAQ on our website at
<http://www.mycotaxon.com>

RECENT BOOKS FROM MYCOTAXON

Prices include surface mail shipment (*for more books see* **www.mycotaxon.com/books.html**)
Mycotaxon, Ltd., P. O. Box 264, Ithaca, NY 14851-0264, U.S.A.

Fungi of China, by S. C. Teng.

Mycotaxon, Ltd. 1996. Hardbound, xiv + 586 pp., 426 illustrations, map, portrait, index, 8–1/2x11 inches. $79.00.
Airmail price varies by country: enquire. ISBN 0-930845-05-6.
Edited by Richard P. Korf, with a foreword by Teng's daughter, Rosaline Z. Deng.
Proceeds from sales go to the Teng family.

Special for *Mycotaxon* subscribers: deduct $20 from regular price!

What reviewers say about Teng's Fungi of China:

"... one of the most valuable books on the fungi of China ever published in English." —*Mycologia* 90: 1091-1092, 1998.

"... a great big blue uncompromising brick of a book ... an instant classic. Anyone interested in identification of fungi, particularly in Asia, should get this book immediately. Mycology owes another debt to Dick Korf for his sympathetic and intelligent editing of this superb work." —*Bibliography of Systematic Mycology* 10(6): xlviii-xlix. 1998.

"The book is important for any researcher interested in the systematic relationships, diversity, and distribution of fungi on a global scale." - *New Zealand Journal of Botany* 35: 265-266. 1977.

Fungi of Northwestern China, edited by Wen-Ying Zhuang.

Mycotaxon, Ltd. 2005. Hardbound, 430 pp., 26 x 18.5 cm. US$40.00 plus postage. ISBN 0-930845-14-5.
(Please ***order direct*** from Wen-Ying Zhuang, PO Box 2714, Beijing 100080, China; zhuangwy@sun.im.ac.cn).

Some 3887 named species belonging to 759 genera of fungi (slime molds, plasmodiophoromycetes, chytrid, oomycetes, zygomycetes, lichens, non-lichenized ascomycetes, anamorphic fungi, rusts, smuts, and the basidiomycetous macrofungi) hitherto known from Gansu, Ningxia, Qinghai, Shaanxi and Xinjiang, China are listed. Useful references, detailed tropical distribution, and hosts or substrates are provided for each species. *Contributing authors:* Shuang-Lin Chen, Lin Guo, Shou-Yu Guo, Ying-Lan Guo, Shu-Xiao Sun, Shu-Xia Wei, Hua-An Wen, Xiao-Qing Zhang,Jian-Yun Zhuang, and Wen-Ying Zhuang.

Higher Fungi of Tropical China, edited by Wen-Ying Zhuang.

Mycotaxon, Ltd. 2001. Cloth bound, 485 pp., 26 x 18.5 cm. US$50.00 plus postage. ISBN 0-930845-13-7.
(Please ***order direct*** from Wen-Ying Zhuang, PO Box 2714, Beijing 100080, China; zhuangwy@sun.im.ac.cn).

Some 5056 named species belonging to 1192 genera of higher fungi hitherto known from tropical China are listed. Useful references, detailed tropical distribution, and hosts or substrates are provided for each species. *Contributing authors:* Lin Gou, Shou-Yu Guo, Ying-Lan Guo, Xiao-Lan Mao, Shu-Xiao Sun, Shu-Xia Wei, Hua-An Wen, Zhi-He Yu, Xiao-Qing Zhang, Jian-Yun Zhuang, and Wen-Ying Zhuang.

A Monograph of the Fungus Genus *Cercospora,* by Charles Chupp

Published by the author, Ithaca. 1953. Hardbound, 667 pp. 222 illustrations, octavo, $25.00. Airmail, $45.00
Proceeds from sales go to the Chupp family.
Still a classic after a half century.

Mycotaxon Cumulative Index for Volumes XLI-LX (1991-1996)

Karen D. Gettelman, Richard P. Korf, Susan C. Gruff & Ian F. Korf
Mycotaxon, Ltd. 1998. Softbound, 296 pp., 6x9 inches. $35.00. Airmail, $55.00. ISBN 0-930845-07-2.

Mycotaxon Cumulative Index for Volumes XXI-XL (1984-1991)

Richard P. Korf & Susan C. Gruff
Mycotaxon, Ltd. 1991. Softbound, 352 pp., 6x9 inches. $30.00. Airmail, $50.00. ISBN 0-930845-01-3.

Mycotaxon Cumulative Index for Volumes I-XX (1974-1984)

Richard P. Korf & Susan C. Gruff
Mycotaxon, Ltd. 1984. Softbound, 232 pp., 6x9 inches. $17.50. Airmail, $37.50.
ISBN 0-930845-00-5.

FUNGAL DIVERSITY RESEARCH SERIES (published in Hong Kong): Mycotaxon, Ltd. now ships rapidly from the U.S. to North American customers. All prices are *postpaid.* Titles include: **Marine Mycology—A Practical Approach,** Kevin D Hyde & Stephen B. Pointing (eds.), $100, 2000. **Genera of Ascomycetes from Palms,** by Kevin D. Hyde, Joanne E. Taylor & Jane Fröhlich, $100, 2000. **Palm Microfungi,** by Jane Frölich & Kevin D. Hyde, $100, 2000. **A World Monograph of *Anthostomellaa,*** by Binsheng Lu & Kevin D. Hyde, $100, 2000. **Checklist of Hong Kong Fungi,** by B.S. Lu, K.D. Hyde, W.H. Ho, H.M. Tsui, M.K.M. Wong, Yanna & D.Q. Zhou, $20, 2000. **Bio-Exploitation of Filamentous Fungi,** Stephen B. Pointing & Kevin D. Hyde (eds.), $100, 2001. **Fungi in Marine Environments,** Kevin D. Hyde (ed.), $80, 2002. **Smut fungi from New Zealand—Fungi of New Zealand volume 2,** by Kálmán Vánky & Eric H.C. McKenzie, $50, 2002. **The Genus *Mycena* in South-Eastern Australia,** by Cheryl A. Grgurinovic, $80, 2002. **Freshwater Mycology,** by C.K.M. Tsui & K.D. Hyde, $80, 2003. **Myxomycetes of New Zealand—Fungi of New Zealand volume 3,** by Stephen L. Stephenson, $50, 2003. **Microfungi of Tropical and Temperate Palms,** by Joanne E. Taylor & Kevin D. Hyde, $80, 2003. **Revision of the genus *Amphisphaeria,*** by Y.Z. Wang, André Aptroot & Kevin D. Hyde, $60, 2004. For details see the Hong Kong University website:

www.hku.hk/ecology/mycology/FDRS/index.htm

Statement of ownership, management, and circulation of Mycotaxon, Required by Act of 12 August 1970: Section 3685, Title 39, United States Code, filed 14 October 1985.

Mycotaxon is published quarterly by Mycotaxon, Ltd., 316 Richard Pl., Ithaca, NY 14850-3129. General Business office: P.O. Box 264, Ithaca, NY 14851-0264. USPS Publication #16-121, ISBN 0093-4666. Editor: Lorelei Norvell, 6720 NW Skyline Blvd., Portland, OR 97229-1309. Business Manager & Contact Person: Richard P. Korf, P.O. Box 264, Ithaca, NY 14851-0264 (phone 607-273-0508). Owner: Mycotaxon, Ltd. Stockholders owning or holding 1 percent or more of the total amount of stock are: Ian F. Korf, 510 Lake Blvd. #166, Davis, CA 95616; Kumiko T. Korf, 316 Richard Pl., Ithaca, NY 14850; Mario T. Korf, 700 Walnut St. #3, San Carlos, CA 94070; Mia K. LeBeau, 1356 N. Ogden Dr., Los Angeles, CA 90046; Noni K. Vidal, 313 Richard Pl., Ithaca, NY 14850.

The known bondholders, mortgagees and other security holders owning or holding 1 percent or more of the total amount of bonds, mortgages, or other securities are: none.

Circulation: Average number of copies printed for each issue during the preceding 12 months: **675**; for single issue nearest to filing date: **675**. Sales through dealers and carriers, street vendors, and counter sales: none. Average number of paid mail subscribers during preceding 12 months, **540**; for single issue nearest to filing date: **534**. Average number of free copies distributed by mail during preceding 12 months, **50**; for single issue nearest to filing date:**51**. Average number of free copies distributed outside the mail during the preceding 12 months, **2**; for single issue nearest filing date, **2**. Percent paid and/or requested circulation during the preceding 12 months, **91.216%**; for the single issue nearest to filing date: **90.971%**. Average number of copies distributed during the preceding 12 months, **592**; for single issue nearest to filing date, **587**. Average number of undistributed copies for office use during the preceding 12 months, **83**; for single issue nearest filing date, **88**.

I certify that the statements made by me above are correct and complete.

Signed, *Richard P. Korf,* Business Manager
September 22, 2005.

Mycotaxon is published quarterly during the periods of January–March, April–June, July–September, and October-December by **Mycotaxon, Ltd.**, 316 Richard Pl., Ithaca, NY 14850–0264. USPS Publication # 16-121, ISSN # 0093-4666. Periodical postage paid at Ithaca, NY, and at additional mailing offices. Subscription rates for 2005: In U.S. and possessions, one year, $330; reduced rate for personal subscribers, one year, $150. Foreign subscriptions, add $40 for IMEX air mail.

POSTMASTER!
Send address changes to **Mycotaxon, Ltd.**
P.O. Box 264, Ithaca, NY 14851-0264 USA

[Content continued from inside front cover]

Paecilomyces parvosporus, a new species with its relatives from Yunnan Province, China **Yanfeng Han, Zongqi Liang, Huali Chu & Jichuan Kang** 357
Notes on *Otidea* from Xinjiang, China **Wen-Ying Zhuang** 365
Book reviews and notices **David L. Hawksworth** 371

Indices & Information

Nomenclatural novelties proposed in volume 94 393
Author index for volume 94 394
Reviewers for volume 94 398
MYCOTAXON online resources—Index to Fungous and Lichen Taxa, cumulative indices, distributional checklists, past volumes online, information for authors & reviewers, search features 399
Errata 400
MYCOTAXON Instructions to authors 401
Recent books from MYCOTAXON 412

EDITORS OF MYCOTAXON

Lorelei L. Norvell, Editor-in-Chief
Pacific Northwest Mycology Service
6720 NW Skyline Boulevard
Portland, OR 97229, U.S.A

Shaun R. Pennycook, Nomenclature Editor
PennycookS@LandcareResearch.co.nz
Manaaki Whenua Landcare Research
Auckland, New Zealand

David L. Hawksworth
Book Review Editor
MycoNova, Calle Aguila 12
Colonia La Maliciosa, Mataelpino
ES-28411 Madrid, Spain

Karen D. Gettelman
Index Editor
510 Lake Blvd., Apt. 166
Davis, CA 95616
U.S.A.

Grégoire L. Hennebert
French Language Editor
32 Rue de l'Élevage
B-1340 Ottignies - LLN
Belgium

MYCOTAXON is a quarterly, peer-reviewed journal devoted to mycological taxonomy and nomenclature. All articles are reviewed by specialists prior to submission. Publication is open to everyone. Papers may be in English or in French. Summaries in those or any additional languages desired by the authors are given for longer articles. Printing is on high quality, acid-free book paper. Authors prepare their own *text-ready* files after having received critical comments from pre-submission reviewers before undergoing additional presubmission review by the Nomenclature Editor. *Instructions to Authors* (Mycotaxon **90:** 495–507, 2004) and *Guidelines for Reviewers* (Mycotaxon **78:** 539-540, 2001) are updated on our website at <www.mycotaxon.com>.

SUBSCRIPTION INFORMATION

Current volumes contain at least 400 pages. All subscriptions are on an annual basis, with four quarterly volumes each year. Personal subscriptions are available at a substantially reduced rate for those who agree not to deposit their copies in a library other than their personal one within 3 years of receipt. Address **all** orders to the MYCOTAXON ORDER DEPARTMENT, P.O. Box 264, Ithaca, NY 14851-0264 USA, or your agent. Prices and availability of back volumes are posted on-line at <www.mycotaxon.com> or can be provided on request by Email from <info@mycotaxon.com>. Mycotaxon may be obtained on a journal-exchange basis. Proposals for such exchanges from journals, institutions, or individuals with difficulty in obtaining foreign currency should be addressed to the Order Department.

The annual subscription rates for 2005 & 2006 (four volumes annually) are

	USA	**Foreign (IMEX air assist)**
REGULAR (multi-user)	$330	$370
PERSONAL (individual)	$150	$190

All back volumes [except volumes 22, 24, 34(2), 35, 41, 54, and 64, currently out of print] are available at $35 each by surface mail, $55 each by air mail. Volumes 1, 38, 39, 43 and 46 were reprinted in 2001; the original printing of volume 1, part 1 is provided, with parts 2 and 3 reprinted.

CUMULATIVE INDICES

Printed versions of the *Mycotaxon Cumulative Indices* for Volumes 1-20 (ISBN 0-930845-00-5), Volumes 21-40 (ISBN 0-930845-01-03), and Volumes 41-60 (ISBN 0-930845-07-02) are available (see descriptions at <www.mycotaxon.com> and order there or through your local bookstore's listings under "Books in Print"). An online *Cumulative Index to Taxa in Volumes 61-ff.* and the *Cumulative Author Index to Volumes 61-ff.* are posted at <www.mycotaxon.com>.

AVAILABILITY IN MICROFILM, PHOTOCOPY, & ELECTRONIC VERSIONS

MYCOTAXON is also partially available in *microfilm* from National Archive Publishing Company <www.napubco.com>. *Tear sheets* or *photocopies* of individual articles may be obtained from ISI Document Solution <www.isidoc.com>. Many back volumes are available as free downloads through Cyberliber <www.cybertruffle.org.uk/cyberliber/index.htm> under 'Catalogues & Journals.'

CONTACTING MYCOTAXON'S EDITOR-IN-CHIEF BY E-MAIL OR FAX

To reach the Editor-in-Chief regarding manuscripts, E-mail to <editor@mycotaxon.com> or Fax to Lorelei L. Norvell at +503.297.3296.

CONTACTING MYCOTAXON'S ORDER DEPARTMENT BY E-MAIL OR FAX

To reach the Order Department for information or placing orders, you may E-mail to <info@mycotaxon.com> or you may Fax to Orders, Mycotaxon, Ltd., May–October at +607.273.4357, and November–April at +727.321.1460.

VISIT MYCOTAXON ON THE WORLD WIDE WEB AT <www.mycotaxon.com>

K
192.
5
.06
n./Mar.

MYCOTAXON

THE INTERNATIONAL JOURNAL OF FUNGAL TAXONOMY & NOMENCLATURE

Volume 95 *January–March 2006*

CONTENTS

Taxonomic studies on Ustilaginomycetes—26 **Kálmán Vánky** 1

Kalmusia amphiloga comb. nov. on *Bambusa* **Ove E. Eriksson** 67

A new species and new records of *Rhytismatales* from Taiwan **Cheng-Lin Hou, Roland Kirschner & Chee-Jen Chen** 71

Macromycetes of Pinacate and Great Altar Desert biosphere reserve **Martín Esqueda, Martha Coronado, Alfonso Sánchez, Evangelina Pérez-Silva & Teófilo Herrera** 81

A new species and a new record of *Lycoperdaceae* from India **Dipika Bisht, J.R. Sharma, Hanns Kreisel & Kanad Das** 91

Notes on the type, synonyms, and other specimens of the balansioid fungus, *Nigrocornus scleroticus* **Malcolm J. Ryley** 97

Rick´s species revision: *Mitremyces zanchianus* versus *Calostoma zanchianum* **Iuri G. Baseia, Vagner G. Cortez & Francisco D. Calonge** 113

Taxonomic studies on Indian *Phellinus* s.l. species: parsimony analysis using morphological characters **Prasad Lamrood & Aristóteles Góes-Neto** 117

Paecilomyces verticillatus, a new species isolated from soil in China **Zhu Li, Yanfeng Han & Zongqi Liang** 133

A monograph of the genus *Cookeina* (Ascomycota, *Pezizales, Sarcoscyphaceae*) **Teresa Iturriaga & Donald H. Pfister** 137

A new nematode-trapping hyphomycete of *Arthrobotrys* **Weifeng Hu, Yan Li, Minghe Mo & Keqin Zhang** 181

Hyphodontia tubuliformis, a new species from Taiwan **Sheng-Hua Wu** 185

Calculating minimum sample sizes for taxonomic measurements: examples using Gäumann's *Peronospora* spore data **Andrew J. Hamilton & James H. Cunnington** 189

Tricholoma borgsjoeënse, a new species from a boreal coniferous forest in Fennoscandia **S. Jacobsson, S. Muskos & E. Larsson** 195

A new variety of *Meliola thalliformis* from Brazil **Dartanhã J. Soares, Douglas Ferreira Parreira & Robert Weingart Barreto** 201

Russula in Himalaya 2: Four new taxa **Kanad Das, S.L. Miller & J.R. Sharma** 205

The distinction between *Menegazzia cincinnata* and *M. valdiviensis* (*Parmeliaceae*) **Mónica T. Adler, Susana Calvelo & John A. Elix** 217

NATS truffle and truffle-like fungi 13: *Tuber quercicola* and *T. whetstonense,* new species from Oregon, and *T. candidum* redescribed **Jonathan L. Frank, Darlene Southworth & James M. Trappe** 229

Two new species of *Parmotrema* (*Parmeliaceae,* Lichenized Ascomycota) from Brazil **Cristine G. Donha & Sionara Eliasaro** 241

[Content continues inside front cover]

ISSN 0093-4666 MYXNAE 95: 1–350 (2006)

For subscription details, availability on microform, and availability of articles as photocopies or tear sheets, see back cover

[Content continued from front cover]

Russula siamensis: a new species of annulate *Russula* from Thailand
S. Yomyart, J. Piapukiew, R. Watling, A.J.S. Whalley & P. Sihanonth 247
Embellisia oxytropis, a new species isolated from *Oxytropis kansuensis* in China
Qi Wang, Hideyuki Nagao, Yu-ling Li, Hong-sheng Wang & Makoto Kakishima 255
Some hyphomycetes from Brazil. Two new species of *Brachydesmiella,* two new combinations for *Repetophragma,* and new records
Rafael F. Castañeda-Ruiz, Luís Fernando Pascholati Gusmão, Gabriela Heredia Abarca & Masatoshi Saikawa 261
Russula in Himalaya 3: A new species of subgenus *Ingratula*
Kanad Das, J.R. Sharma & N.S. Atri 271
Wallemia, a genus newly recorded from China **Guangyu Sun, Miao Zhang, Rong Zhang, Huining Ma & Mark L. Gleason** 277
Phialophora sessilis, a lithobiont fungus **Giuseppe Caretta, Solveig Tosi, Eduardo Piontelli & G.S. de Hoog** 281
Revision of *Termitomyces* species originally described from China
B.-H. Tang, T.-Z. Wei & Y.-J. Yao 285
Two new species of *Marasmius* (Basidiomycota, *Marasmiaceae*) from Brazil
Carla Puccinelli & Marina Capelari 295
Geastrum hirsutum: a new earthstar fungus with a hairy exoperidium
I.G. Baseia & F.D.Calonge 301
Tricholoma lavendulophyllum, a new species from Yunnan, China
Fu-Qiang Yu, Yun Wang & Pei-Gui Liu 305
Changes and additions to the North American lichen mycota – V
Kerry Knudsen & James C. Lendemer 309
Four new lichens from Turkey **Kenan Yazıcı & Alı Aslan** 315
Two new species in the genus *Morchella* (*Pezizales, Morchellaceae*) from China
Shu-Hong Li, Yong-Chang Zhao, Hong-Mei Chai & Ming-Hui Zhong 319
Notes on some species of the lichen genus *Lecidea* from India
D.K. Upreti, S. Nayaka & M.P. Andreev 323
Contributions to the macrofungi of Bolu and Düzce Provinces, Turkey
Dursun Yağız, Ahmet Afyon, Muhsın Konuk, Stephan Helfer 331
First isolation of *Aphanomyces frigidophilus* (*Saprolegniales*) in Europe
Isabel Ballesteros, María P. Martín & Javier Diéguez-Uribeondo 335

Indices & Information

Nomenclatural novelties proposed in volume 95 *341*
Author index *343*
Reviewers *347*
Errata *348*
From the Editor-in-Chief *349*

MYCOTAXON

THE INTERNATIONAL JOURNAL OF FUNGAL TAXONOMY & NOMENCLATURE

A. R. MANN LIBRARY
JUN 27 2006
ITHACA, NY 14853

Volume 95, 2006

COMPLETE IN ONE VOLUME

CONSISTING OF VI + 350 PAGES INCLUDING FIGURES

EDITOR-IN-CHIEF

Lorelei L. Norvell
editor@mycotaxon.com
Pacific Northwest Mycology Service
6720 NW Skyline Boulevard
Portland, Oregon 97229-1309 USA

ASSOCIATE EDITORS

NOMENCLATURE EDITOR

Shaun R. Pennycook
PennycookS@LandcareResearch.co.nz
Manaaki Whenua Landcare Research
Auckland, New Zealand

FRENCH LANGUAGE EDITOR

Grégoire Hennebert
32 Rue de l'Élevage
B-1340 Ottignies – LLN, Belgium

BOOK REVIEW EDITOR

David L. Hawksworth
MycoNova, Calle Aguila 12
Colonia La Maliciosa, Mataelpino
ES-28411 Madrid, Spain

INDEX EDITOR

Karen D. Gettelman
510 Lake Blvd., Apt. 166
Davis, CA 95616 U.S.A.

EDITORIAL ADVISORY BOARD

Donald H. Pfister, Cambridge, Massachusetts, USA (1997-2007), Chair
Gary J. Samuels, Beltsville, Maryland, USA (1997-2006), Past Chair
Wen-Ying Zhuang, Beijing, China (2003-2008)
Carol A. Shearer, Urbana, Illinois, USA (1998-2009)
Shaun Ross Pennycook, Auckland, New Zealand (2005-2010)
Seppo Huhtinen, Turku, Finland (2006-2011)

Published by
MYCOTAXON, LTD, P. O. BOX 264
Ithaca, NY 14581-0264, USA

www.mycotaxon.com

Printed in the United States of America

© Mycotaxon, Ltd, 2006

MYCOTAXON

VOLUME NINETY-FIVE TABLE OF CONTENTS

Taxonomic studies on Ustilaginomycetes—26 **Kálmán Vánky** 1

Kalmusia amphiloga comb. nov. on *Bambusa* **Ove E. Eriksson** 67

A new species and new records of *Rhytismatales* from Taiwan
Cheng-Lin Hou, Roland Kirschner & Chee-Jen Chen 71

Macromycetes of Pinacate and Great Altar Desert biosphere reserve
Martín Esqueda, Martha Coronado, Alfonso Sánchez, Evangelina Pérez-Silva & Teófilo Herrera 81

A new species and a new record of *Lycoperdaceae* from India
Dipika Bisht, J.R. Sharma, Hanns Kreisel & Kanad Das 91

Notes on the type, synonyms, and other specimens of the balansioid fungus, *Nigrocornus scleroticus* **Malcolm J. Ryley** 97

Rick´s species revision: *Mitremyces zanchianus* versus *Calostoma zanchianum*
Iuri G. Baseia, Vagner G. Cortez & Francisco D. Calonge 113

Taxonomic studies on Indian *Phellinus* s.l. species: parsimony analysis using morphological characters **Prasad Lamrood & Aristóteles Góes-Neto** 117

Paecilomyces verticillatus, a new species isolated from soil in China
Zhu Li, Yanfeng Han & Zongqi Liang 133

A monograph of the genus *Cookeina* (Ascomycota, *Pezizales, Sarcoscyphaceae*)
Teresa Iturriaga & Donald H. Pfister 137

A new nematode-trapping hyphomycete of *Arthrobotrys*
Weifeng Hu, Yan Li, Minghe Mo & Keqin Zhang 181

Hyphodontia tubuliformis, a new species from Taiwan **Sheng-Hua Wu** 185

Calculating minimum sample sizes for taxonomic measurements: examples using Gäumann's *Peronospora* spore data
Andrew J. Hamilton & James H. Cunnington 189

Tricholoma borgsjoeënse, a new species from a boreal coniferous forest in Fennoscandia **S. Jacobsson, S. Muskos & E. Larsson** 195

A new variety of *Meliola thalliformis* from Brazil
Dartanhã J. Soares, Douglas Ferreira Parreira & Robert Weingart Barreto 201

Russula in Himalaya 2: Four new taxa **Kanad Das, S.L. Miller & J.R. Sharma** 205

The distinction between *Menegazzia cincinnata* and *M. valdiviensis* (*Parmeliaceae*)
Mónica T. Adler, Susana Calvelo & John A. Elix 217

NATS truffle and truffle-like fungi 13: *Tuber quercicola* and *T. whetstonense,* new species from Oregon, and *T. candidum* redescribed
Jonathan L. Frank, Darlene Southworth & James M. Trappe 229

Two new species of *Parmotrema* (*Parmeliaceae,* Lichenized Ascomycota) from Brazil **Cristine G. Donha & Sionara Eliasaro** 241

Russula siamensis: a new species of annulate *Russula* from Thailand **S. Yomyart, J. Piapukiew, R. Watling, A.J.S. Whalley & P. Sihanonth** 247

Embellisia oxytropis, a new species isolated from *Oxytropis kansuensis* in China **Qi Wang, Hideyuki Nagao, Yu-ling Li, Hong-sheng Wang & Makoto Kakishima** 255

Some hyphomycetes from Brazil. Two new species of *Brachydesmiella,* two new combinations for *Repetophragma,* and new records **Rafael F. Castañeda-Ruiz, Luís Fernando Pascholati Gusmão, Gabriela Heredia Abarca & Masatoshi Saikawa** 261

Russula in Himalaya 3: A new species of subgenus *Ingratula* **Kanad Das, J.R. Sharma & N.S. Atri** 271

Wallemia, a genus newly recorded from China **Guangyu Sun, Miao Zhang, Rong Zhang, Huining Ma & Mark L. Gleason** 277

Phialophora sessilis, a lithobiont fungus **Giuseppe Caretta, Solveig Tosi, Eduardo Piontelli & G.S. de Hoog** 281

Revision of *Termitomyces* species originally described from China **B.-H. Tang, T.-Z. Wei & Y.-J. Yao** 285

Two new species of *Marasmius* (Basidiomycota, *Marasmiaceae*) from Brazil **Carla Puccinelli & Marina Capelari** 295

Geastrum hirsutum: a new earthstar fungus with a hairy exoperidium **I.G. Baseia & F.D.Calonge** 301

Tricholoma lavendulophyllum, a new species from Yunnan, China **Fu-Qiang Yu, Yun Wang & Pei-Gui Liu** 305

Changes and additions to the North American lichen mycota – V **Kerry Knudsen & James C. Lendemer** 309

Four new lichens from Turkey **Kenan Yazıcı & Alı Aslan** 315

Two new species in the genus *Morchella* (*Pezizales, Morchellaceae*) from China **Shu-Hong Li, Yong-Chang Zhao, Hong-Mei Chai & Ming-Hui Zhong** 319

Notes on some species of the lichen genus *Lecidea* from India **D.K. Upreti, S. Nayaka & M.P. Andreev** 323

Contributions to the macrofungi of Bolu and Düzce Provinces, Turkey **Dursun Yağız, Ahmet Afyon, Muhsın Konuk, Stephan Helfer** 331

First isolation of *Aphanomyces frigidophilus* (*Saprolegniales*) in Europe **Isabel Ballesteros, María P. Martín & Javier Diéguez-Uribeondo** 335

Indices & Information

Nomenclatural novelties proposed in volume 95 341
Author index 343
Reviewers 347
Errata 348
From the Editor-in-Chief 349

PUBLICATION DATE FOR VOLUME NINETY-FOUR

MYCOTAXON *for* OCTOBER–DECEMBER, VOLUME 94 (1–412 + I–VI)
was issued on May 11, 2006

MYCOTAXON

Volume 95, pp. 1–65 January–March 2006

Taxonomic studies on Ustilaginomycetes - 26

Kálmán Vánky

vanky.k@cityinfonetz.de
Herbarium Ustilaginales Vánky (HUV)
Gabriel-Biel-Str. 5, D-72076 Tübingen, Germany

Abstract—Fifteen new species of smut fungi are described: *Doassansiopsis tomasii, Microbotryum afromontanum, Pilocintractia adrianae, Sporisorium andropogonis-chinensis, S. andropogonis-eucomi, S. andropogonis-pumili, S. distachyum, S. ingoldii, S. livingstoneanum, S. scholzii, Ustanciosporium virginianum, Ustilago gabonensis, U. penniseti-purpurei, U. pentaschistidis, U. trichogena.* New names proposed are: *Sporisorium andropogonis-gabonensis* and *S. clintonianum.* New combinations are: *Heterodoassansia hygrophilae, Sporisorium andropteri, S. sanctae-catharinae, S. semisagittatum, S. sorghastri, S. zilligii, Ustilago jardineae.* Taxa placed in synonymy are: *Entyloma lavrovianum, Leucocintractia leucodermoides, Pericladium flavesci, Sorosporium chloridicola, S. platense, Sphacelotheca rhaphidis, Tilletia cynodontis, Ustilago andropogonis-hirtifolii, U. liebenbergii.* A lectotype is designated for *Ustilago chloridicola.* Keys are given to the species of *Pilocintractia* and to the smut fungi of *Andropogon, Chloris, Jardinea, Leptochloa, Nymphaea, Phacelurus, Rhytachne,* and *Sorghastrum.*

Key words—nomenclature, smut fungi, synonym, taxonomy

The smut fungi of *Andropogon* (*Poaceae*)

Andropogon L. is a genus with about 100 species in the tropics belonging to the subfam. *Panicoideae,* tribe *Andropogoneae,* subtribe *Andropogoninae* (Clayton & Renvoize, 1986:349). Numerous smut fungi have been described on it. Zundel (1930), in his monograph of the *Ustilaginales* attacking *Anropogon*, recognised 76 species. Since 1930, the classification of both the grasses and the smut fungi has changed considerably. Many "*Andropogon*" species in Zundel's paper do not belong to this genus any more, and many smut fungi do not belong to the genus under which they were treated by Zundel. On *Andropogon* s. str. 32 species of smut fungi could be distinguished, including 6 new species. At the same time, *Sorosporium platense* was found to be a synonym of *Sporisorium andropogonis*, and *Ustilago andropogonis-hirtifolii* a synonym of *Sporisorium stuhlmannii.*

The smut fungi of *Andropogon* were compared also with the six smut fungi described on the closely related *Schizachyrium* (comp. Vánky, 2003:36-43; 2004a:74). These six species proved to be distinct from the species on *Andropogon*. However, several smut fungi occur on both *Andropogon* and *Schizachyrium* species. The recognised smut fungi of *Andropogon* are:

1. *Jamesdicksonia brunkii* (Ellis & Galloway) J. Walker & R.G. Shivas, 1998:1212.

Ustilago brunkii Ellis & Galloway, March 1890:31. — *Tolyposporella brunkii* (Ellis & Galloway) G.P. Clinton, 1902:147. — *Tilletia brunkii* (Ellis & Galloway) Durán, 1972:2572 (invalid comb., no basionym and place of publication indicated; ICBN 33.2). — Type on *Andropogon argenteus* (= *A. ternarius*), USA, Texas, Brazos Co., College Station, 1890, H.S. Jennings, BPI 192453.

Ustilago apiculata Ellis & Galloway, in Jennings, May 1890:29. — Type on *Andropogon saccharoides*, USA, Texas, Brazos Co., College Station, 1890, H.S. Jennings, BPI 192453; isotype BPI 157287! (syn. by Clinton, 1902:129, confirmed).

Sori on the adaxial surface of the leaf sheaths, forming striae fusing into a blackish-brown, agglutinated to granular-powdery coat of spore masses, showing through the outer surface of the leaf sheaths, between the veins, as pale lead coloured striae of various length. *Spores* globose, subglobose to ovoid (in some specimens to subpolyhedrally irregular), extremely variable in size, 10-18 x 10-20(-24) µm in diameter, yellow to dark reddish-brown; wall 3-8 µm thick, composed of a homogenous, uniform endospore of 1-2 µm, and a multilayered, smooth exospore, sometimes with a short, hyaline papilla. (Spore measurements made in unheated lactophenol because in heated lactophenol or in water the spore wall and the spores swell considerably). *Spore germination* (Durán, 1972:2572, fig. 17; Bauer et al., 2001:420, fig. 9) results in holobasidia producing apically 4-8, allantoid, fusiform, symmetrical, septate basidiospores which do not fuse but germinate apically producing ballistoconidia. Asymmetrical ballisto basidiospores are also produced on the basidia on sterigmata.

On *Andropogon barbinodis* Lag. (*Bothriochloa barbinodis* (Lag.) Herter; *A. saccharoides* Sw. var. *barbinodis* (Lag.) Hack.; *A. saccharoides* Sw. var. *leucopogon* (Nees) Hack.), *A bicornis* L., *A. gerardii* Vitman, *A. hirtiflorus* (Nees) Kunth var. *pubiflorus* (Nees) Kunth, *A. perforatus* Trin. ex Fourn., *A. saccharoides* Sw. (*A. torreianus* Steud.; *Bothriochloa saccharoides* (Sw.) Rydb.), *A. ternarius* Michx. (*A. argenteus* Ell.; *A. argyraeus* Schult.), *Dichanthium sericeum* (R. Br.) A. Camus, *D. sericeum* subsp. *polystachyum* (Benth.) B.K. Simon, *Schizachyrium hirtiflorum* Nees (*Andropogon hirtiflorus* (Nees) Kunth; *S. sanguineum* (Retz.) Alston var. *hirtiflorum* (Nees) S.L. Hatch); Australia, N., C. & S. America, West Indian Antilles.

2. *Jamesdicksonia caribensis* M. Piepenbring, 2003:220.

Type on *Andropogon bicornis*, Dominican Republic, Azua Prov., Cordillera Central, La Cumbre, 3.III.1930, E.L. Ekman, S; isotype BPI 192463!

Sori forming striae on the leaves and leaf sheaths, linear, 0.2-0.5 x 1-5 mm, or longer by confluence, initially covered by the epidermis which ruptures longitudinally disclosing the black, agglutinated to granular spore mass. *Spores* very variable in shape and size, globose, ellipsoidal, lemon-shaped or irregular, often with appendages, 7-14 x 8-16(-18) µm, light to medium dark olivaceous-brown; wall uneven, 1.5-6.5 µm thick, including a thin, uniform endospore, and a multilayered, smooth exospore of variable thickness.

On *Andropogon bicornis* L.; West Indian Antilles (Dominican Rep., Puerto Rico).

Jamesdicksonia caribensis differs from *J. brunkii* in having smaller and more irregular spores and a thinner spore wall.

3. *Macalpinomyces ovariicolopsis* (Vánky) Vánky, 2002a:427.

Sporisorium ovariicolopsis Vánky, 2000:203. — Type on *Andropogon gayanus*, Zimbabwe, Matabeleland North Prov., 12 km N of Lusulu, alt. 1010 m, 16.III.1999, C. & K. Vánky, HUV 18903!; isotypes BPI 746891, IMI 380467, S.

Sori in sessile spikelets, infecting only a few in the inflorescence, leaving intact the outermost floral envelopes, lemon-shaped or obovoid with acute or subacute tip, 3-5 x 5-10 mm, covered by a thick, initially green later brown peridium of host and fungal origin which ruptures and opens irregularly disclosing the agglutinated to semi-powdery, dark brown mass of spores intermixed with sterile cells. The sori start to develop at the basal part of the ovaries. The distal part of the seeds may persist on the top of young sori as an acute tip. *Spores* single when mature, subglobose, ovoid or ellipsoidal, (5.5-)6.5-8(-9.5) x (6.5-)7-11 µm, yellowish-brown; wall even, 1-1.5 µm thick, prominently, rather densely echinulate. *Sterile cells* in loose, irregular groups or in short rows, single cells subglobose, ovoid or irregular, with one or two flattened sides, 5-9 x 6-11(-14) µm, hyaline; wall thin, c. 0.5 µm, smooth.

On *Andropogon gayanus* Kunth; Africa (Malawi, Zambia, Zimbabwe).

4. *Sporisorium andropogonis* (Opiz) Vánky, 1985:113.

Uredo (*Ustilago*) *andropogonis* Opiz, 1824:43 (as '*andropogi*'). — *Sphacelotheca andropogonis* (Opiz) Bubák, 1912:25. — *Cintractia andropogonis* (Opiz) Kochman, 1936:75. — Type on *Andropogon angustifolius* (= *Dichanthium ischaemum*), Czech Rep., Dablizerberg [Ďáblice] Mt., near Prague, F.M. Opiz.

For taxonomic synonyms such as *Ustilago ischaemi* Fuckel, *U. cylindrica* Peck, *U. bothriochloae-intermediae* Padwick, *Sphacelotheca chloridis* Mundk., *S. heteropogonis-triticei* L. Ling, *S. bothriochloae* Y.C. Wang, *Sorosporium baluchistani* S. Ahmad, see Vánky, 2004b:226-227.

Sorosporium platense Hirschhorn, 1941:348 (as '*platensis*'). — *Sphacelotheca platensis* (Hirschh.) Hirschhorn, 1986:129. — Type on *Andropogon saccharoides*, Argentina, Buenos Aires, Fac. de Agron. y Veter., XII.1936, E. Hirschhorn, Herb. Hirschhorn 363; isotype LPS 3057! (syn. nov.).

Sori usually destroying the entire inflorescence, rarely confined to the spikelets, cylindrical or bifurcate at their distal part, 1-10 mm wide, 1.5-7 cm long, partly hidden by the terminal leaf sheath, initially covered by a well developed, yellowish-brown peridium which ruptures irregularly and flakes away disclosing the dark brown, semiagglutinated to powdery mass of spore balls, spores and groups of sterile cells surrounding a simple or ramified, irregular columella of the length of the sorus, the remnants of the floral axis and branches. Infection systemic. *Spore balls* loose, subglobose, ellipsoidal, oblong or irregular, 20-100 x 40-160 µm, dark reddish-brown, composed of tens of spores which separate very easily. *Spores* single when mature, globose, ovoid, ellipsoidal to slightly irregular, (6.5-)7-10 x 7.5-11 µm, light olivaceous-brown; wall even, 0.5-1 µm thick, finely, densely punctate-verruculose, spore profile smooth to wavy, in SEM spores minutely echinulate, between the spines, finely and densely verruculose. *Spore germination* (Brefeld, 1883:96, Pl. XI, figs. 1-2; Vánky, Deml & Oberwinkler, 1988:185) results in four-celled basidia on which lateral and terminal basidiospores are produced. *Sterile cells* in irregular groups or chains among the spores and also forming the peridium, globose to irregularly polygonal, flattened on contact sides, 6-16(-22) µm long, subhyaline to yellow tinted, with numerous droplets, collapsed in old specimens; wall even, 0.5-1 µm thick, smooth.

On *Andropogon arctatus* Chapm., *A. barbinodis* Lag., *A. chinensis* (Nees) Merr., *A. imperatoides* (Hack.) Lillo, *A. liebmannii* Hack., *Andropogon* sp., *Bothriochloa bladhii* (Retz.) S.T. Blake (*B. glabra* (Roxb.) A. Camus), *B. caucasica* (Trin.) C.E. Hubb., *B. decipiens* (Hack.) C.E. Hubb., *B. ewartiana* (Domin) C.E. Hubb., *B. intermedia* (R. Br.) A. Camus, *B. pertusa* (L.) A. Camus, *Dichanthium annulatum* (Forssk.) Stapf, *D. insculptum* (A. Richard) Clayton (*Andropogon pubescens* Vis.), *D. ischaemum* (L.) Roberty (*Andropogon angustifolius* Sibth. & Sm.; *A. ischaemum* L.; *B. ischaemum* (L.) Keng), *Diheteropogon amplectens* (Nees) Clayton (*Andropogon amplectens* Nees), *Heteropogon contortus* (L.) P. Beauv. ex Roem. & Schult., *H. triticeus* (R. Br.) Stapf ex Craib; cosmopolitan (Europe, Africa, Asia, Australia, N., C. & S. America).

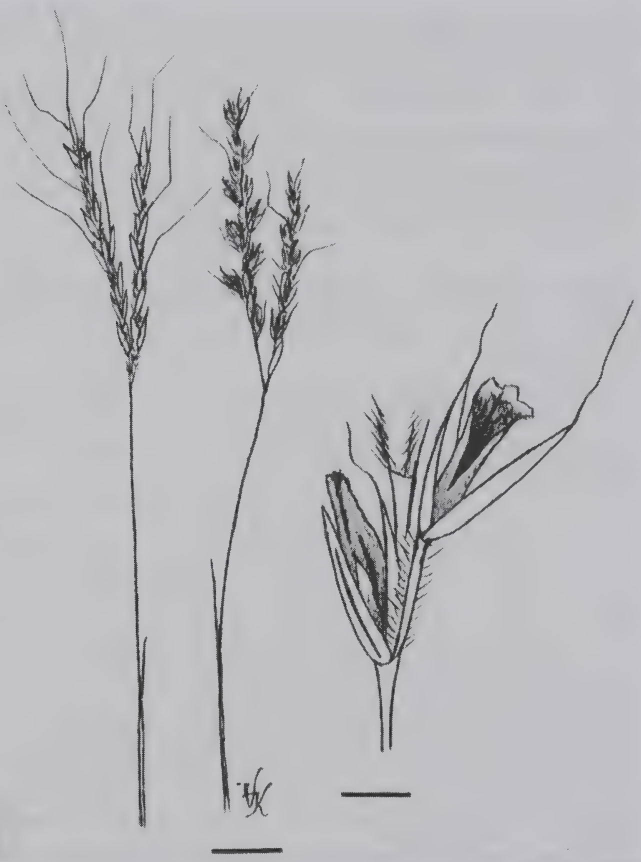


Fig. 1. Sori of *Sporisorium andropogonis-chinensis* in all sessile and pedicelled spikelets of *Andropogon chinensis* (type). Habit, and enlarged two infected spikelets. To the left a healthy inflorescence. Bars = 1 cm for habit, and 2 mm for the detail drawing.

5. ***Sporisorium andropogonis-chinensis*** Vánky, **sp. nov.**

Typus in matrice Andropogon chinensis *(Nees) Merr., Zambia, Eastern Prov., 407 km ENE urbe Lusaka, 14°16'54" S, 31°37'41" E, alt. 1070 m.s.m., 18.IV.2001, leg. C., T. & K. Vánky. Holotypus in Herbario Ustil. Vánky, HUV 21063!*

Sori *in spiculis et sessilibus et pedicellatis eiusdem inflorescentiae, longe ellipsoidales, cca. 1 x 3-5 mm, involucris floralibus plus-minus obtecti, peridio pallide brunneo cooperti, quo irregulariter rupto massam atrobrunneam, semiagglutinatam usque granuloso-pulveream glomerulorum sporarum et sporarum columellam compactam, attenuatam, saepe apice breviter bifurcatam circumdantium ostendentes.* Glomeruli sporarum *globosi, ovoidei usque late ellipsoidales, 20-30 x 25-40(-50) µm, flavidobrunnei, e 10-50(?) sporis leviter dissociabilibus compositi.* Sporae *dimorphae: externae earum globosae, subglobosae, ellipsoidales usque parum irregulares, 6,5-9 x 7,5-10,5 µm, flavidobrunneae; pariete inaequali, 0,5-1 µm crasso, in superficie externa crassiore, moderate dense prominenter verruculoso, imago obliqua eorum serrata. Sporae internae forma et magnitudine sporis externis cca. aequales, pallide flavidobrunneae; pariete aequali, 0,2-0.3 µm crasso, levi.* Cellulae steriles *absentes.*

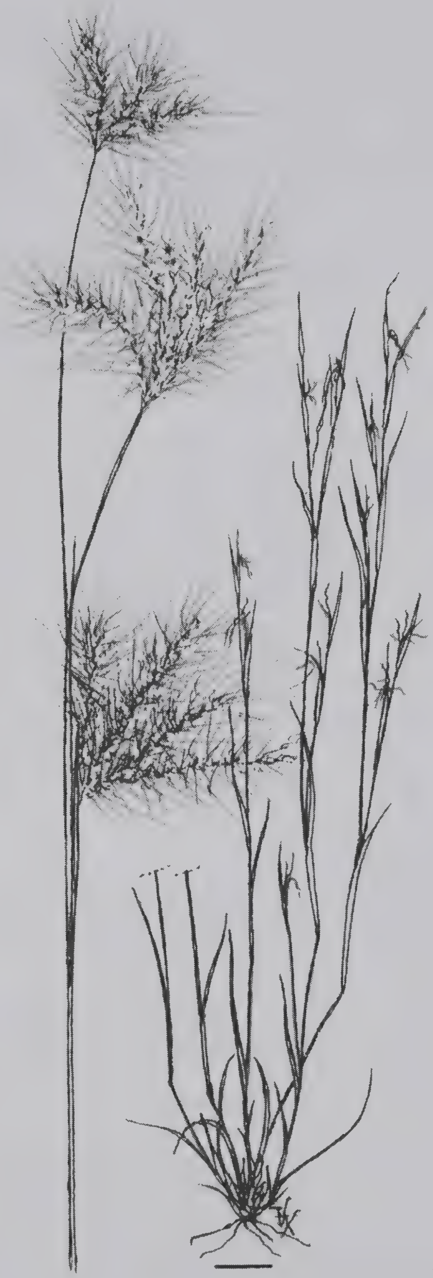
Sori (Fig. 1) in all, sessile and pedicelled spikelets of an inflorescence, long ellipsoidal, c. 1 x 3-5 mm, more or less hidden by the floral envelopes and covered by a pale brown peridium which ruptures irregularly disclosing the dark brown, semiagglutinated to granular-powdery mass of spore balls and spores surrounding a stout, narrowing columella, often with a shortly bifurcate tip. *Spore balls* (Figs. 4, 5) globose, ovoid to broadly ellipsoidal, 20-30 x 25-40(-50) µm, yellowish-brown, composed of 10-50(?) spores which separate easily. *Spores* (Figs. 4, 5) dimorphic, outer spores globose, subglobose, ellipsoidal to slightly irregular, 6.5-9 x 7.5-10.5 µm, yellowish-brown; wall uneven, 0.5-1.5 µm, thicker on the free surface which is moderately densely, prominently verrucose, spore profile serrate. Inner spores about the shape and size of the outer spores, pale yellowish-brown; wall even, 0.2-0.3 µm thick, smooth. *Sterile cells* absent.

On *Andropogon chinensis* (Nees) Merr.; C. Africa. Known only from the type collection.

6. ***Sporisorium andropogonis-eucomi*** Vánky, **sp. nov.**

Typus in matrice Andropogon eucomus *Nees, South Africa, Mpumalanga Prov., 9 km NE urbe Graskop, via R534, 24°51'50" S, 30°51'21" E, alt. 1590 m.s.m., 22.I.1997, leg. C. & K. Vánky. Holotypus in Herbario Ustil. Vánky, HUV 21060; isotypi in BPI, BRIP, IMI, PREM.*

Sori *inflorescentiam totam destruentes, cylindrici, cca. 1 x 6-12 mm, vagina folii supremi obtecti, propter hoc tantum apicibus eorum protrudentes, primo peridio flavidobrunneo cooperti, quo irregulariter rupto massam semiagglutinatam usque granuloso-pulveream, nigram glomerulorum sporarum et columellarum nonnullarum filiformium, tenuium ostendentes.* Glomeruli sporarum *subglobosi, ovoidei, ellipsoidales, elongati vel parum irregulares, (20-)25-40 x (25-)30-50 µm, atro-rubrobrunnei, e sporis (2-)4-15(-20?) pressu separantibus compositi.* Sporae *in glomerulis magnis, dimorphae, externae earum subglobosae, ovoideae, ellipsoidales usque parum irregulares, 12-15 x 13,5-18.5(-20) µm, atro-rubrobrunneae; pariete inaequali, 0,8-2,5(-3) µm crasso, in superficie externa verrucis magnis moderate dense distributis, in lateribus contactis leniter verrucoso. Sporae internae subpolyedricae, magnitudine eadem sporarum externarum cca. aequales, sed colore pallidiores; pariete tenuiori, aequali vel inaequali, leniter punctato-verruculoso.* Cellulae steriles *absentes.*



2

Fig. 2. Sori of *Sporisorium andropogonis-eucomi* in all inflorescences of *Andropogon eucomus* (type). To the left some healthy inflorescences.

Bar = 1 cm.

Sori (Fig. 2) destroying the whole inflorescence, cylindrical, c. 1 x 6-12 mm, hidden by the uppermost leaf sheath from which only the tip of the sori protrudes, at first covered by a yellowish-brown peridium which ruptures irregularly disclosing the black, semiagglutinated to granular-powdery mass of spore balls and several filiform, slender columellae. *Spore balls* (Figs. 6, 7) subglobose, ovoid, ellipsoidal, elongate or slightly irregular, (20-)25-40 x (25-)30-50 µm, dark reddish-brown, composed of (2-)4-15(-20?) spores that separate by pressure. *Spores* (Figs. 6, 7) in larger balls dimorphic, outer spores subglobose, ovoid, ellipsoidal to slightly irregular, 12-15 x 13.5-18.5(-20) µm, dark reddish-brown; wall uneven, 0.8-2.5(-3) µm thick, with moderately densely situated, large warts on the free surface, finely verrucose on the contact sides. Inner spores subpolyhedral, about the size of the outer spores, paler coloured; wall thinner, even or uneven, finely punctate-verruculose. *Sterile cells* absent.

On *Andropogon eucomus* Nees; S. Africa. Known only from the type collection.

Sporisorium andropogonis-eucomi is closest to *S. pseudomaranguense* (type on *Andropogon* sp., South Africa), from which it differs especially by fewer spores in the balls and larger spores with thicker spore wall.

7. ***Sporisorium andropogonis-gabonensis*** Vánky, **nom. nov.**

Replacing *Sorosporium congoense* L. Ling, Lloydia 16:186, 1953a (not *Sporisorium congense* (Syd. & P. Syd.) Vánky, type on *Hyparrhenia diplandra*). — Type on *Andropogon gabonensis,* Congo, N. Dembo, VI.1906, H. Vanderyst B32, BR 339! Paratypes on *Andropogon gabonensis,* Congo, N. Dembo, 23.VI.1908, H. Vanderyst, BR 275; Congo, Kinshasa, 4.VI.1906, H. Vanderyst, BR 1326.

Sori in the spikelets protruding beyond the enveloping glumes, cylindrical, c. 1.5 x 7-18 mm, initially covered by a greyish-brown peridium which ruptures from its apex disclosing the dark brown, semiagglutinated to powdery mass of loose spore balls and spores surrounding several filiform columellae. *Spore balls* globose, ovoid, ellipsoidal, oblong or irregular, 30-75 x 30-100 µm, dark reddish-brown, composed of tens of spores which separate easily. *Spores* globose, subglobose, ovoid, ellipsoidal to slightly irregular, 6-9 x 7-10 µm, pale reddish-brown; wall uneven, 0.5-1 µm thick, sparsely, prominently, low verrucose, spore profile smooth to finely wavy. *Sterile cells* not seen.

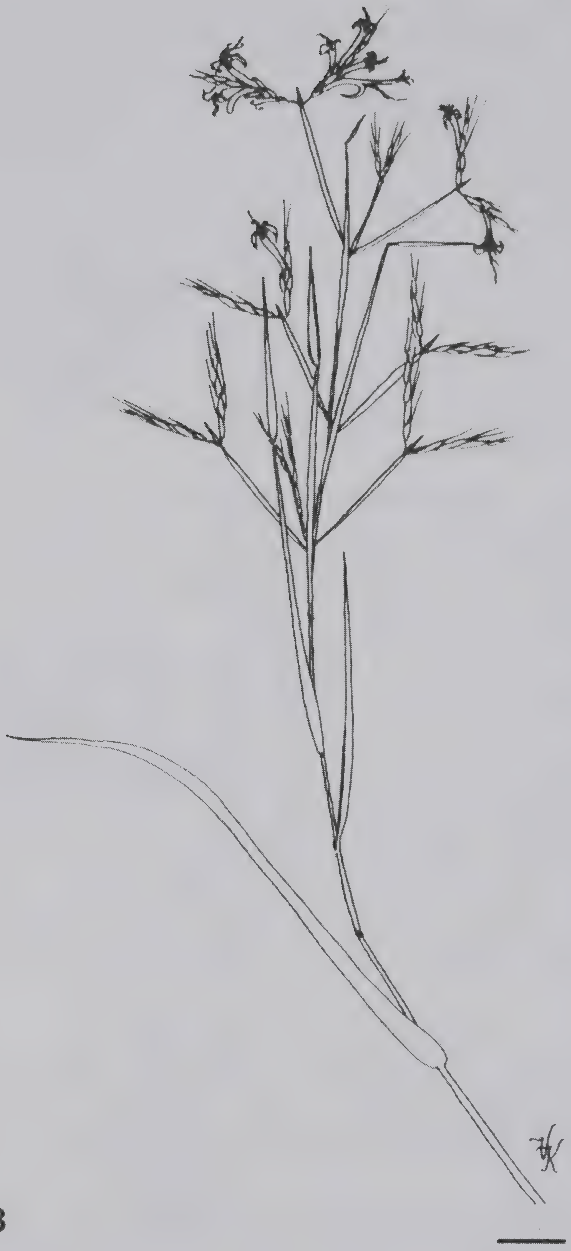
On *Andropogon gabonensis* Stapf; C. Africa (Congo).

The specific epithets *congoense* and *congense* are confusingly similar and are considered as orthographic variants (homonyms), hence the proposed new name *andropogonis-gabonensis* (ICBN Art. 53.3).

8. ***Sporisorium andropogonis-pumili*** Vánky, **sp. nov.**

Typus in matrice Andropogon pumilus *Roxb., India, Maharashtra State, Pune (Poona), 2 km SW of M.A.C.S., 18°34' N, 73°58' E, alt. cca. 600 m, 23.X.1992, leg. C. & K. Vánky. Holotypus in Herbario Ustil. Vánky, HUV 21041! Paratypus: 15 km W urbe Pune, National Defence Academy, 18°34' N, 73°50' E, alt. cca. 720 m, 20.X.1992, leg. C. & K. Vánky, HUV 21040!*

Sori *in spiculis nonnullis et sessilibus et pedicellatis inflorescentiae eiusdem, cylindrici, saepe parum arcuati, 0,5-1 x 4-10 mm, involucris floralibus partim obtecti, primo peridio crasso, cremeo cooperti, quo ab apice eius irregulariter rupto massam glomerulorum sporarum nigrescentibrunneam primo agglutinatam, serius granulosopulveream, columellam centralem longam, filiformem, simplicem circumdantem ostendentes.* Glomeruli sporarum *forma et magnitudine varii, subpolyedrice irregulares, elongati,*



3

Fig. 3. Sori of *Sporisorium andropogonis-pumili* in some sessile and pedicelled spikelets of *Andropogon pumilus* (type). Bar = 1 cm.

rarius ovoidei vel ellipsoidales, 30-100 x 40-130 µm, atro-rubrobrunnei usque subopaci, permanentes, e pluribus decem sporis pressu difficiliter separabilibus compositi. Sporae *dimorphae: externas earum subglobosae, ellipsoidales usque subpolyedrice irregulares, 9-12,5 x 10,5-14,5 µm, uniformiter flavidobrunneae; pariete sine ornamentis aequaliter cca. 0,5 µm crasso, pariete libero cum ornamentis 1,5-3 µm crasso: etiam sub LM conspicue cum verrucis dense dispositis, filiformibus, 1-2,5 µm longis, in medio superficiei liberae altissimis, pariete contacto cca. 0,5 µm crasso apparenter levi, sub SEM valde leniter verruculoso. Sporae internae rotundae, subpolyedriciter irregulares, magnitudine cellulis externis cca. aequales, pallide flavidobrunneae; pariete aequali, tenui (cca. 0,3 µm), levi.* Cellulae steriles *absentes.*

Sori (Fig. 3) in some sessile and pedicelled spikelets of an inflorescence, cylindrical, often slightly bent, 0.5-1 x 4-10 mm, partly hidden by the floral envelopes, initially covered by a thick, cream coloured peridium which ruptures irregularly from its apex disclosing the blackish-brown, first agglutinated later granular-powdery mass of spore balls surrounding a long, filiform, simple, central columella. *Spore balls* (Figs. 8, 9) varying in shape and size, subpolyhedrally irregular, elongated, more rarely ovoid or ellipsoidal, 30-100 x 40-130 µm, dark reddish-brown to subopaque, permanent, composed of tens of spores separating with difficulty by pressure. *Spores* (Figs. 8, 9) dimorphic, outer spores subglobose, ellipsoidal to subpolyhedrally irregular, 9-12.5 x 10.5-14.5 µm, uniformly yellowish-brown; the wall without ornamentation is uniformly c. 0.5 µm thick, with ornamentation the free wall is 1.5-3 µm thick, as seen by LM provided with densely situated, filiform, 1-2.5 µm long warts, highest in the middle of the free surface, contact walls apparently smooth, in SEM very finely verruculose. Inner spores rounded, subpolyhedrally irregular, about the size of the outer spores, pale yellowish-brown; wall even, thin (c. 0.3 µm), smooth. *Sterile cells* absent.

On *Andropogon pumilus* Roxb.; S. Asia (India).

Sporisorium andropogonis-pumili is closest to *S. everhartii*, from which it differs especially by the morphology of the outer spores. In *S. everhartii* these are 8-15 µm long, medium dark reddish-brown with paler and darker areas, caused by the uneven, 0.5-1.5 (-2.5) µm thick wall, and the warts of the free surface are up to only 0.5 µm high.

9. *Sporisorium andropogonis-schirensis* (L. Ling) Vánky, 2005a:262.

Sphacelotheca andropogonis-schirensis L. Ling. 1953a:181. — Type on *Andropogon schirensis*, Congo, Bandundu, II.1914, H. Vanderyst 3555, BR 1349; isotypes in BPI 193953, K, HUV 18276!

Sori in all spikelets of an inflorescence, ellipsoidal, 1-1.5 x 2-3.5 mm, evident between the spreading glumes, initially covered by a brown, rather thick peridium which ruptures from its apex disclosing the dark brown, semiagglutinated to powdery mass of loose spore balls, spores and groups of sterile cells, surrounding a stout, tapering columella. *Spores* single when mature, subglobose, ellipsoidal, ovoid to slightly irregular, 6.5-9 x 7-10.5(-11) µm, yellowish-brown; wall even, 0.5-0.7 µm thick, finely, rather sparsely to moderately densely verruculose-echinulate, spore profile in LM smooth, in SEM, between the small spines finely, densely verruculose. *Sterile cells* in irregular groups, collapsed in old specimens, single cells 7-15.5 µm long, hyaline; wall thin (c. 0.5 µm), smooth.

On *Andropogon appendiculatus* Nees, *A. schirensis* A. Rich.; C. & S. Africa. (Congo, South Africa).

10. *Sporisorium andropogonis-tectorum* (L. Ling) Vánky, 2004a:106.

Ustilago andropogonis-tectorum L. Ling, 1953b:152. — Type on *Andropogon tectorum*, Sierra Leone, Hill Station, 27.VII.1941, F.C. Deighton M2302, IMI 10966; isotype BPI 157098. Paratype on *Andropogon tectorum*, Nigeria, 1936, J. West 69, IMI 44426; isoparatype HUV 17809!

Sori destroying the whole young floral shoots, transforming each into an elongated, somewhat curved, up to 20 cm long, whip-like structure, initially covered by the epidermis which flakes away disclosing the olivaceous-brown, semiagglutinated to powdery mass of spores intermixed with sterile cells, produced around a stout, narrowing columella with longitudinal furrows. *Spores* globose, subglobose, often flattened on one side, 7.5-9.5 x 8-10.5(-12) µm, yellowish-brown; wall slightly thinner on one side, 0.4-0.8 µm thick, finely, densely verrucose-echinulate, spore profile wavy to finely serrulate. *Sterile cells* in irregular groups, single sells 8-16 µm long, subhyaline, usually collapsed; wall c. 1 µm thick, smooth.

On *Andropogon tectorum* Schum.; Africa.

11. *Sporisorium bicornis* (Henn.) Vánky 1996:103.

Ustilago bicornis Henn., in Pazschke, 1896a:50. — *Sphacelotheca bicornis* (Henn.) Zundel, 1930:140. — Type on *Andropogon bicornis*, Brazil, Campo Bello, IV.1894, E. Ule 2079.

Sori in considerably swollen spikelets of a few racemes of an inflorescence, appearing as witches' brooms. Single sori long-cylindrical, 0.5-1 x 10-30 mm, curved, first covered by a thick, brown, fungal peridium which ruptures from its distal part disclosing the chocolate-brown, powdery mass of loose spore balls and spores surrounding a well developed central columella. *Spores* single when mature, globose, subglobose, ovoid, ellipsoidal or slightly irregular, 6-9.5 x 6.5-11(-12) µm yellowish-brown; wall even, 0.5-1 µm thick, moderately densely punctate to echinulate, spore profile smooth to finely wavy. *Sterile cells* few among the spores, varying in shape and size, 4-12 µm long, hyaline, thin-walled (c. 0.5 µm), smooth, forming shorter or longer chains.

On *Andropogon bicornis* L.; S. America (Brazil, Colombia).

In its appearance, *Sporisorium bicornis* resembles *S. holwayi* from which it differs especially by spore measurements, which in *S. holwayi* are 9-14 x 10-16(-18) µm.

12. *Sporisorium braziliense* (Zundel) M. Piepenbring, 2002a:54 (as '*braziliensis*').

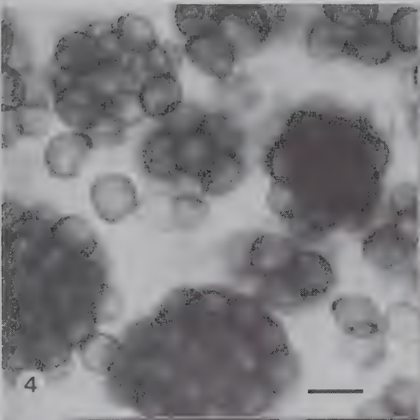
Sphacelotheca braziliensis Zundel, 1931:297. — Type on *Andropogon leucostachyus*, Brazil, State Minas Gerais, Serra do Cipo, 28.III.–1.IV.1925, A. Chase 9168, BPI 177294!; isotype BPI 177295!

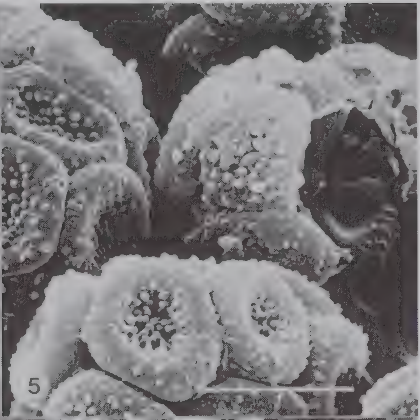
Sphacelotheca macrotrichis Zundel, 1933:354 (as '*macrothricis*'). — Type on *Andropogon macrothrix*, Brazil, State Minas Gerais, Uberlandia (Uberabinha), 15.III.1930, A. Chase 11253½, BPI 178070!; isotype BPI 178069! (syn. by Piepenbring, 2003:90).

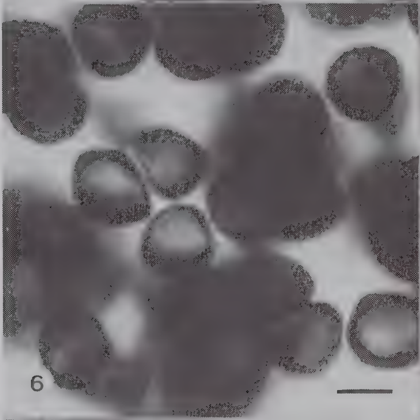
Figs. 4, 5. Spore balls and spores of *Sporisorium andropogonis-chinensis* on *Andropogon chinensis*, in LM and in SEM (type).

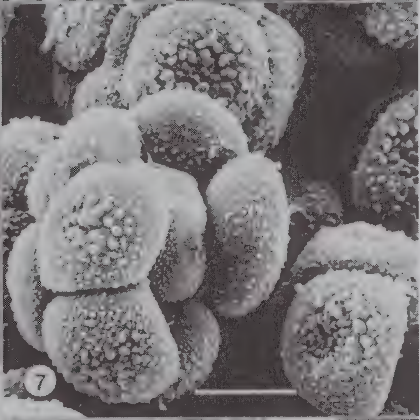
Figs. 6, 7. Spore balls and spores of *Sporisorium andropogonis-eucomi* on *Andropogon eucomus*, in LM and in SEM (type).

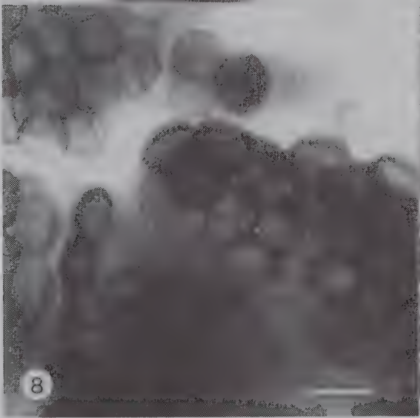
Figs. 8, 9. Spore balls and spores of *Sporisorium andropogonis-pumili* on *Andropogon pumilus*, in LM and in SEM (type). Bars = 10 µm.

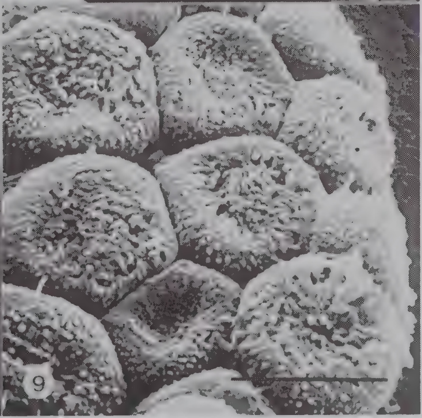

4


5


6


7


8


9

Sori in all spikelets of an inflorescence, cylindrical, 0.8-1.5 x 3-5 mm, first covered by a thin greyish peridium which ruptures irregularly disclosing the dark brown, powdery mass of loose spore balls and spores, surrounding a flagelliform central columella. *Spores* single when mature, globose, ovoid, ellipsoidal, slightly flattened on one side (6.5-9 µm wide), 8.5-12 x 9-14 µm, light to medium dark yellowish-brown; wall 0.5-1.2 µm thick, thinner on the flattened side, moderately densely verrucose-echinulate, spore profile wavy. *Sterile cells* among the spores not seen.

On *Andropogon leucostachyus* Kunth, *A. macrothrix* Trin., *Andropogon* sp.; S. America (Brazil).

13. *Sporisorium culmiperdum* (J. Schröt.) Vánky, 1993:40.

Ustilago culmiperda J. Schröt. in Hennings, 1896b:212. — *Sphacelotheca culmiperda* (J. Schröt.) G.P. Clinton, in Zundel, 1930:143. — Type on *Andropogon bicornis*, Brazil, Santa Catarina, near Joinville, V.1883, E. Ule 1627, BPI 159932; isotypes BPI 194444, 195257.

Sori in the whole inflorescence, long linear, 1-2 mm wide, 3-7 cm long, partly hidden by the terminal leaf sheath, first covered by a dark brown peridium which flakes away revealing a blackish-brown, dusty spore mass surrounding a well-developed, often bent columella. *Spores* globose, subglobose to ovoid, 12-18 x 14-19 µm, medium to dark reddish-brown with a paler, rounded area (germ pore) of 5-7 µm diameter; wall 1.5-2 µm thick, thinner at the paler areas, surface finely, densely verrucose-echinulate. *Spore germination* (Piepenbring, 1996:97) results in four- to several-celled basidia, producing basidiospores. *Sterile cells* absent.

On *Andropogon bicornis* L., *A. gerardii* Vitman var. *hondurensis* R.W. Pohl, *A. glomeratus* (Walter) Britton, Stern. & Poggenb.; N., C. & S. America.

14. *Sporisorium distachyum* Vánky, **sp. nov.**

Typus in matrice Andropogon distachyos *L., Ethiopia, Arsi Reg., 241 km SE urbe Addis Abeba, 77 km S pag. Asela, 07°22'57.3" N, 39°15'08.9" E, alt. 2985 m.s.m., 4.XI.2004, leg. T. & K. Vánky. Holotypus in Herbario Ustil. Vánky, HUV 20909; isotypi in Vánky, Ust. exs. no. 1286. Paratypus in matrice* Andropogon abyssinicus *Fresen., Ethiopia, Arsi Reg., 162 km SE urbe Addis Abeba, 2 km N pag. Asela, 07°59'14.9" N, 39°08'39.6" E, alt. 2345 m.s.m., 3.XI.2004, leg. T. & K. Vánky, HUV 20908; isoparatypi in Vánky, Ust. exs. no. 1287.*

Sori *in spiculis omnibus et sessilibus et pedicellatis inflorescentiae eiusdem, organa interna floralia destruentes, inter involucra distantia floralia prominentes, cilindrici, cca. 1 x 2-4 mm, primo peridio atrobrunneo cooperti, quo mature ab apice rupto massam glomerulorum sporarum nigram, semiagglutinatam usque pulveream et sporarum atque cellularum sterilium columellam brevem, simplicem, centralem circumdantem ostendentes.* Glomeruli sporarum *laxi, mature dissoluti.* Sporae *in maturitate earum singulae, globosae, subglobosae, ovoideae, ellipsoidales usque parum subpolyedrice irregulares, 9-12 x 10,5-13,5 µm, mediocriter atro-flavidobrunneae, cum poro germinationis leviter pallidiore, rotundo, diametro cca. 3 µm; pariete parum inaequali, 0,5-1(-1,5) µm crasso, ad poros germinationis tenuiore, leniter, dense verrucoso-echinulato, imago obliqua sporarum levis usque leniter serrulata.* Cellulae steriles *in catervis irregularibus, cellulae singulae forma et magnitudine variae, globoideae, ellipsoidales usque plerumque irregulares, latere uno vel lateribus nonnullis deplanatis, 4,5-14,5 x 6-16 µm, hyalinae; pariete aequali, 0,5-1 µm crasso, levi.*

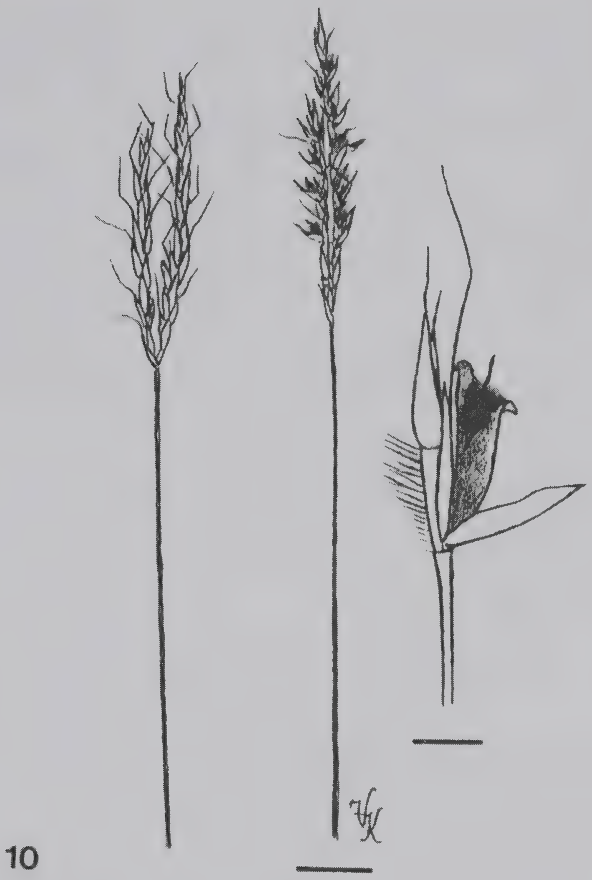


Fig. 10. Sori of *Sporisorium distachyum* in all sessile and pedicelled spikelets of *Andropogon distachyos* (type). Habit, and enlarged an infected spikelet. To the left a healthy inflorescence. Bars = 1 cm for habit, and 3 mm for the detail drawing.

Sori (Fig. 10) in all, both sessile and pedicelled spikelets of an inflorescence, destroying the inner floral organs, showing between the spreading floral envelopes, cylindrical, c. 1 x 2-4 mm, first covered by a dark brown peridium which early ruptures from its apex, disclosing the black, semiagglutinated to powdery mass of spore balls, spores and sterile cells surrounding a short, simple, central columella. *Spore balls* (Figs. 13, 14) loose, early disintegrating. *Spores* (Figs. 13, 14) single when mature, globose, subglobose, ovoid, ellipsoidal to subpolyhedrally slightly irregular, 9-12 x 10.5-13.5 µm, medium dark yellowish-brown, with a slightly paler, rounded germ pore of c. 3 µm diameter; wall slightly uneven, 0.5-1(-1.5) µm thick, thinner at the germ pores, finely, densely verrucose-echinulate, spore profile smooth to finely serrulate. *Sterile cells* (Figs. 13, 14) in irregular groups, single cells variable in shape and size, globoid, ellipsoidal to usually irregular, with one or several flattened sides, 4.5-14.5 x 6-16 µm, hyaline; wall even, 0.5-1 µm thick, smooth.

On *Andropogon abyssinicus* Fresen., *A. distachyos* L.; Africa (Ethiopia).

15. *Sporisorium ellisii* (G. Winter) M. Piepenbring, 2003:103.

Sorosporium ellisii G. Winter, 1883a:2 (Jan.); 1883b:7 (Jan.). — Lectotype (design. by Jackson, 1908:148) on *Andropogon virginicus*, USA, New Jersey, Newfield, X.1880, J.B. Ellis, NY; isolectotypes in Ellis, N. Amer. Fgi. no. 1099, BPI 179797.

Sori destroying the whole inflorescence, long cylindrical, 1-2 x 10-50 mm, more or less hidden by leaf sheaths, initially covered by a pale brown, well-developed peridium which ruptures irregularly at maturity disclosing the blackish-brown, granular-powdery mass of spore balls and spores surrounding a long, thin columella often with bifurcate tip. Sori rarely confined to the spikelets only. *Spore balls* subglobose, ellipsoidal, oblong to irregular, 30-80 x 40-100 µm, dark reddish-brown, composed of tens of spores which separate easily by pressure. *Spores* subglobose, ellipsoidal to usually subpolyhedrally slightly irregular, 11-15 x 12-18.5(-20) µm, reddish-brown with irregular paler and darker areas; wall uneven, 1-2.5 µm thick, including an even, pale endospore and a pigmented, unevenly thickened, densely, prominently low verruculose-echinulate exospore, spore profile wavy to finely serrulate. *Sterile cells* not seen.

On *Andropogon elliottii* Chapm., *A. glomeratus* (Walter) Britton, Stern. & Poggenb., *A. virginicus* L., *Andropogon* sp., *Schizachyrium scoparium* (Michx.) Nash (*Andropogon scoparius* Michx.); N. America (USA).

Sporisorium ellisii is closest to *S. guaraniticum* (Speg.) Vánky, type on *Schizachyrium condensatum*, Paraguay, in which the spores are dimorphic, with smooth inner spores, the spore wall is thicker (2.5-4 µm) and more finely ornamented.

16. *Sporisorium everhartii* (Ellis & Galloway) M. Piepenbring, 1999:462.

Sorosporium everhartii Ellis & Galloway, 1890:32. — *Tolyposporium everhartii* (Ellis & Galloway) Dietel, in Engler & Prantl, 1897:14. — Type on *Andropogon virginicus*, USA, New Jersey, Newfield, X.1887, coll. N. A. F. 2265b, BPI 179946.

Sori in the sessile spikelets of all or of some spikelet pairs of an inflorescence, narrow cylindrical, 0.5-1 x 10-15(-20) mm, protruding between the outermost floral envelopes, first covered by a pale brown peridium which ruptures irregularly from its apex disclosing the blackish-brown, agglutinated to granular-powdery mass of spore balls surrounding a long, filiform, flattened central columella of the length of the sorus. The sori ripen from their apex which may be already empty of spores whereas their basal part is still unripe. *Spore balls* varying in shape and size, globose, ovoid, ellipsoidal, oblong to irregular, 30-100 x 50-130 µm, dark reddish-brown, composed of tens of firmly united spores which separate by hard pressure. *Spores* dimorphic. Outer spores ellipsoidal, tangentially elongated, slightly subpolyhedrally irregular or subcuneiform, 7-12 x 8-15 µm, medium dark reddish-brown with paler and darker areas, according to the unevenly thickened wall; wall 0.5-1.5 µm or up to 2.5 µm thick at the angles, free surface coarsely, moderately densely verrucose-echinulate, warts up to 0.5 µm high, spore profile wavy to serrulate. Inner spores rounded subpolyhedrally irregular, about the size of the outer spores, pale yellowish-brown; wall even or slightly uneven, c. 0.5 µm thick, smooth. *Spore germination* (Durán, 1987:65; Piepenbring, 1999:465) results in 4-7-celled, mononucleate basidia giving rise to ellipsoidal, mononucleate basidiospores.

Sterile cells not seen.

On *Andropogon brachystachyus* Chapm., *A. floridanus* Scribner, *A. gerardii* Vitman, *A. glomeratus* (Walter) Britton, Stern. & Poggenb. (*A. macrourus* Michx.), *A. microstachyus* Desv. ex Hamilt., *A. tener* (Nees) Kunth, *A. ternarius* Michx., *A. urcatus* Muhl., *A. virginicus* L., *A. virginicus* var. *hirsutior* (Hack.) Hitchc., *Schizachyrium hirtiflorum* Nees (*Andropogon hirtiflorus* (Nees) Kunth), *S. scoparium* (Michx.) Nash (*Andropogon scoparius* Michx.); N. America (USA, Mexico, Cuba).

Reports of this smut from Africa (comp. Zundel, 1938:304; 1953:59) refer to other smut fungi. That on *Hyparrhenia ruprechtii* (= *Hyperthelia dissoluta*), from S. Africa, is *Sporisorium ischaemoides* (Henn.) Vánky, PREM 7770! That on "*Andropogon diplandrus* Hack." (= *Hyparrhenia diplandra* (Hack.) Stapf), from Congo, is certainly one of the several *Sporisorium* species on this host plant, known from Africa. "*Sorosporium everhartii* on *Andropogon ischaemum* L." from South Africa (PREM 7770, BPI 179880) represents *Sporisorium ischaemoides* on *Hyperthelia dissoluta* (Nees ex Steud.) Clayton.

17. *Sporisorium fastigiatum* Vánky, 2000:206.

Type on *Andropogon fastigiatus*, Zimbabwe, Matabeleland North Prov., 25 km SE of Binga, alt. c. 690 m, 15.III.1999, C. & K. Vánky, HUV 18910!; isotypes BPI 746885 and in Vánky, Ust. exs. no. 1066. Paratype on *Andropogon fastigiatus*, Matabeleland North Prov., c. 50 km NW of Lusulu, alt. c. 970 m, 16.III.1999, C. & K. Vánky, HUV 18911!

Sori in all sessile and pedicelled spikelets of the inflorescence, completely destroying them, ovoid, 1-2 x 2-4 mm, first covered by a thick, pale yellowish-brown peridium which bears remnants of floral envelopes. At maturity, the peridium ruptures in several places disclosing the semi-agglutinated, blackish-brown mass of spores and sterile cells surrounding a stout, central columella, usually with short lateral branches. Rarely, the sori may comprise the whole inflorescence or a part of it. *Spores* when young in irregular, loose balls, single when mature, subglobose, ellipsoidal, usually rounded, subpolyhedrally irregular, 8-11 x 9-13.5 μm, reddish-brown; wall even or slightly uneven, 0.5-0.8 μm thick, apparently smooth to finely, densely punctate or punctate-echinulate, spore profile smooth to very finely serrulate. *Sterile cells* in compact, rounded or elongated groups, 15-25 x 20-35 μm, these may be agglutinated in short chains; single cells irregular, subpolyhedral with flattened sides, rarely ovoid, 5-13 μm long, subhyaline with homogenous content; wall 0.5-1 μm thick, smooth.

On *Andropogon angustatus* (Presl) Steud., *A. fastigiatus* Swartz (*Diectomis fastigiata* (Swartz) Kunth); Africa (Guinea, Zimbabwe), C. & S. America (Nicaragua, Venezuela).

18. *Sporisorium gayanum* Vánky & C. Vánky, in Vánky, 2000:205.

Type on *Andropogon gayanus*, Zimbabwe, Matabeleland North Prov., 12 km N of Lusulu, alt. c. 1010 m, 16.III.1999, C. & K. Vánky, HUV 18899!; isotypes BPI, IMI 380465, S, and in Vánky, Ust. exs. no. 1065.

Sori in all sessile and pedicelled spikelets of the inflorescence, leaving intact only the outermost floral envelopes, rarely also a few anthers, cylindrical with acute tip, c. 1 x 5-10 mm, covered by a pale yellowish-brown peridium which ruptures early from its apex and curls disclosing the blackish-brown, first agglutinated, later granular-powdery mass of spore balls and 3-6 filiform columellae. In young stage, the peridium often bears remnants of inner floral organs or aborted anthers. *Spore balls* varying in shape and size, broadly ellipsoidal, ovoid, oblong or irregular, 35-90 x 40-120(-140) μm, reddish-brown to opaque, permanent, composed of tens to about a hundred tightly packed spores. *Spores* dimorphic. Outer spores subpolyhedrally irregular, 8-12 x 10.5-14.5 µm, dark reddish-brown with slightly uneven, 1-2 µm thick wall, prominently verruculose-echinulate on the free surface which appears finely serrulate in median view; inner spores subpolyhedrally or polyhedrally irregular, often elongated, tightly packed, the size of the outer spores or somewhat smaller, subhyaline or pale yellowish-brown; wall thin, c. 0.5 µm, even, smooth. *Sterile cells* few, in small groups or chains; single cells subglobose, oblong or irregular, with flattened sides, 5-13 µm long, hyaline; wall thin, c. 0.5 µm, smooth.

On *Andropogon chinensis* (Nees) Merr., *A. gayanus* Kunth; Africa (Malawi, Zambia, Zimbabwe).

19. ***Sporisorium holwayi*** (G.P. Clinton & Zundel) Vánky, 1993:40.

Sphacelotheca holwayi G.P. Clinton & Zundel, in Zundel, 1930:143. — Type on *Andropogon bicornis*, Bolivia, Prov. Sur Yungas, Villa Aspiazu, 31.V.1920, E.W.D. & M.M. Holway, BPI 177831.

Sphacelotheca kellermanii G.P. Clinton & Zundel, in Zundel, 1930:142. — Lectotype on *Andropogon leucostachyus* (det. A. Chase), Guatemala, Los Amates, 15.III.1905, W.A. Kellerman 7601-A (design. by Piepenbring, 2003:108) BPI 178042!; isolectotypes BPI 178041!, 178045! (syn. by Piepenbring, 2003:108).

Sori in groups of spikelets, bunched and forming witches' brooms. One or several witches' brooms of various sizes may occur in the same inflorescence. Individual sori linear, often curved, 0.7-1 x 10-40 mm, initially covered by a greyish-brown peridium which splits longitudinally exposing a blackish-brown mass of spores intermixed with few, irregular groups of sterile cells surrounding a well-developed, horn-shaped, central columella. *Spores* single when mature, subglobose, ovoid to irregularly oblong or subpolyhedral, 9-14 x 10-16(-18) µm, dark reddish-brown; wall even or slightly uneven, c. 1.5 µm thick, evidently, moderately densely echinulate, spore profile serrulate. *Sterile cells* varying in shape and size, smaller than the spores, subhyaline to light yellowish-brown, collapsed with age.

On *Andropogon bicornis* L., *A. leucostachyus* Kunth; C. & S. America.

Sporisorium holwayi differs from the similar *S. bicornis* especially in having much larger, darker, thick-walled spores.

20. ***Sporisorium leucostachys*** (Henn.) M. Piepenbring, 2003:110.

Ustilago leucostachys Henn., in Pazschke, 1896a:50. — *Sphacelotheca leucostachys* (Henn.) Zundel, 1930:144. — Type on *Andropogon leucostachyus*, Brazil, State Minas Gerais, Serra do Itatiaia, II.1894, E. Ule 2096, HBG; isotypes BPI 162412, HUV 17523!

Sori destroying the whole inflorescence, long linear, c. 2 mm wide, 5-7 cm long, partly enclosed by leaf sheaths, first covered by a thick, light brown peridium which ruptures irregularly disclosing the dark brown, granular powdery mass of spore balls surrounding a long, well-developed columella. *Spore balls* subglobose, ovoid, ellipsoidal, oblong to slightly irregular, 30-70 x 40-100 µm, dark reddish-brown, composed of tens of spores which separate by pressure. *Spores* subglobose, ovoid, ellipsoidal to usually rounded, subpolyhedrally slightly irregular, (10.5-)11-13.5 x 12-16 µm, medium dark reddish-brown, with paler and darker areas; wall uneven, 0.5-1.5 µm thick, moderately densely, finely to prominently verrucose, especially on the free surface of the spores, spore profile smooth, wavy or serrulate. *Sterile cells* lacking.

On *Andropogon leucostachyus* Kunth; S. America (Brazil).

21. *Sporisorium livingstoneanum* Vánky, **sp. nov.**

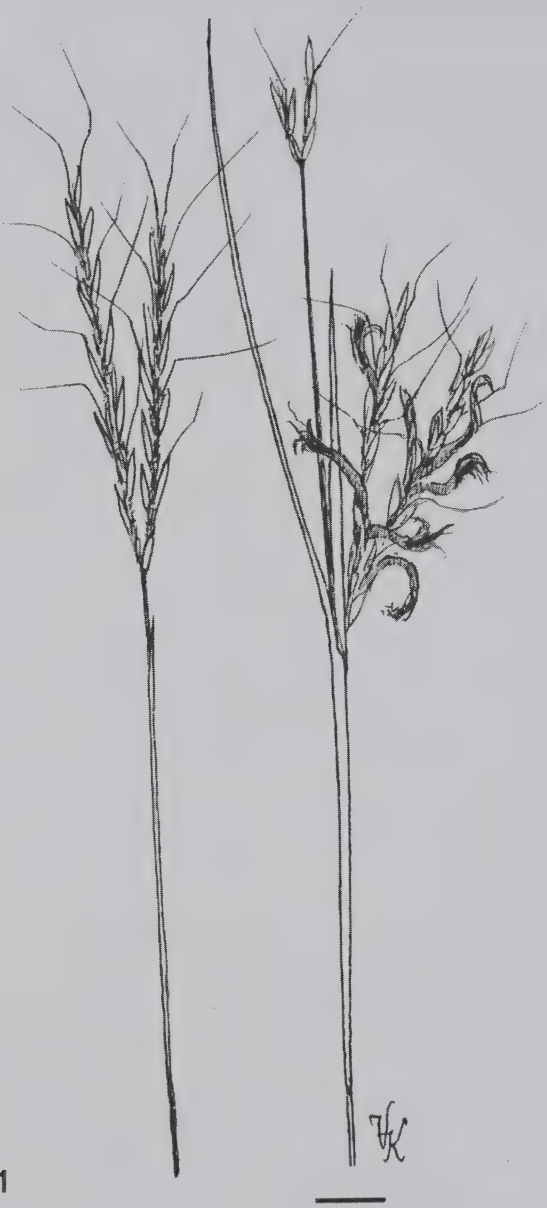
Typus in matrice Andropogon gayanus *Kunth, Zambia, Southern Prov., 10 km N urbe Livingstone, 17°47'45" S, 25°51'10" E, alt. 960 m.s.m., 14.IV.2001, leg. T., C. & K. Vánky. Holotypus in Herbario Ustil. Vánky, HUV 21070!; isotypi in BPI, BRIP et IMI. Paratypi in matrice* Andropogon chinensis *(Nees) Merr., Zambia, Lusaka Prov., 169 km ENE urbe Lusaka, 15°04'10" S, 29°45'22" E, alt. 900 m.s.m., 17.IV.2001, leg. T., C. & K. Vánky, HUV 21072! et Lusaka Prov., 201 km E. urbe Lusaka, 15°00'18" S, 29°58'22" E, alt. 760 m.s.m., 27.IV.2001, leg. C. & K. Vánky, HUV 21073; isoparatypi in BPI, BRIP et IMI.*

Sori *in spiculis nonnullis et sessilibus et pedicellatis inflorescentiae eiusdem, longe cylindrici, arcuati vel torti, 1-1,5 x 5-20(-25) mm, primo peridio crasso, cremeo cooperti, quo ab apice eius irregulariter rupto massam nigrobrunneam, semiagglutinatam usque granuloso-pulveream glomerulorum sporarum et columellarum nonnullarum, filiformium plerumque partim vel omnio in columellam decrescentem, planam, taeniaeformem coalescentium ostendentes.* Glomeruli sporarum *forma et magnitudine varii, globosi, ovoidei, elongati, vel subpolyedrice irregulares, 30-140 x 30-150 μm, glomeruli globoidei diametro 30-140 μm, atro-rubrobrunnei, subopaci vel opaci, permanentes, e sporis pluribus decem usque pluribus centum, pressu valido difficiliter separabilibus compositi.* Sporae *dimorphae, externae earum subglobosae, ovoideae, ellipsoidales usque subpolyedrice parum irregulares, 7,5-10,5 x (8-)9-13(-14) μm, flavido- usque rubrobrunneae; pariete inaequali, 0,5-2 μm crasso, in lateribus contactis tenui, ad angulos et in superficie libera crassissimo, verrucis 0,5(-1) μm altis vel spinis moderate dense distributis inclusis, imago obliqua sporarum superficiei liberae undulata usque leniter serrulata, eadem in lateribus contactis levis. Sporae internae globoideae, ovoideae vel roundato-subpolyedricae, magnitudine sporis externis cca. aequales, pallide flavidobrunneae; pariete aequali, cca. 0,4-0,8 μm crasso, levi, sub SEM ruguloso.* Cellulae steriles *absentes.*

Sori (Fig. 11) in some sessile and pedicelled spikelets of an inflorescence, long cylindrical, bent or curled, 1-1.5 x 5-20(-25) mm, first covered by a thick, cream coloured peridium which ruptures irregularly from its apex disclosing the blackish-brown, semiagglutinated to granular-powdery mass of spore balls and several long, filiform columellae, which usually partly or completely fuse into a narrowing, flat, band-like columella. *Spore balls* (Figs. 15, 16) varying in shape and size, globose, ovoid, elongated or subpolyhedrally irregular, 30-140 x 30-150 μm, globoid balls 30-140 in diameter, dark reddish-brown, subopaque or opaque, permanent, composed of tens to hundreds of spores which separate with difficultly by hard pressure. *Spores* (Figs. 15, 16) dimorphic, outer spores subglobose, ovoid, ellipsoidal to subpolyhedrally slightly irregular, 7.5-10.5 x (8-)9-13(-14) μm, yellowish- to reddish-brown; wall uneven, 0.5-2 μm thick, thin on the contact sides, thickest at the angles and on the free surface, including the moderately densely situated, 0.5(-1) μm high warts or spines. Spore profile on the free surface wavy to finely serrulate, on the contact sides smooth. Inner spores globoid, ovoid or rounded subpolyhedral, about the size of the outer spores, pale yellowish-brown; wall even, c. 0.4-0.8 μm thick, smooth. *Sterile cells* absent.

On *Andropogon gayanus* Kunth, *A. chinesis* (Nees) Merr.; C. Africa (Zambia).

Etymology: This species is named after the Zambian city Livingstone, close to the Victoria Falls, where this smut was first collected.



11

Fig. 11. Sori of *Sporisorium livingstoneanum* in some sessile and pedicelled spikelets of *Andropogon gayanus* (type). Habit. To the left a healthy inflorescence. Bar = 1 cm.

22. ***Sporisorium mexicanum*** (Vánky) Vánky & Cunnington, in Cunnington, Vánky & Shivas, 2005:98.

Lundquistia mexicana Vánky, 2004c:161. — Type on *Andropogon gerardii*, Mexico, Durango State, 57 km WSW of Durango, Hwy no. 48, alt. 2538 m, 19.XI.2003, T. & K. Vánky, HUV 20498; isotypes in Vánky, Ust. exs. no. 1202. Paratype on *Schizachyrium mexicanum*, Mexico, Mexico State, 18.5 km W of Toluca, Hwy no. 1, alt. 2812 m, 6.XII.2003, C. & K. Vánky, HUV 20526; isoparatypi in BPI and IMI.

Sori on the top of sterile shoots as dark, long, slender, bent bodies composed of numerous vascular bundles and among them spore masses and sterile cells destroying the parenchymatous tissues. At maturity the host tissue disintegrates and the dark brown, semiagglutinated to powdery mass of spores and sterile cells is successively liberated leaving behind a 2-5 mm wide, 15-40 cm long, twisted and curled fascicle of numerous, filiform columellae. *Spores* globose to subglobose, 8-10.5 × 8-11 µm, medium dark yellowish-brown; wall 2-2.5 µm thick including the 1.5-2 µm high, anastomosing warts which form an irregular, labyrinthiform or incompletely and irregularly reticulate pattern. Warts in optical median view acute. *Sterile cells* single, in short chains or in small, irregular groups. Single cells subglobose or ellipsoidal with flattened contact sides, 7-13 µm long, subhyaline to pale yellowish-brown; wall even, c. 0.5 µm thick, smooth. *Spore germination* (Vánky, 2004c:161 + fig. 2) results in 4-celled basidia (often in 3+1 arrangement), measuring 2-2.5 × 15-25 µm. On the basidia, laterally and terminally, ovoid basidiospores measuring 1.5-2.5 × 4-5.5 µm are produced on sterigmata. The basidiospores bud like yeast cells giving rise to yeast colonies.

On *Andropogon gerardii* Vitman, *Schizachyrium mexicanum* (Hitchc.) A. Camus; N. America (Mexico). Known only from the type collections.

23. ***Sporisorium occidentale*** (Seym. ex G.P. Clinton) Vánky & Snets., in Vánky, 1990:270.

Sphacelotheca occidentalis Seymour ex G.P. Clinton, 1902:141, nom. nud.; 1904:389. — Type on *Andropogon furcatus* (= *A. gerardii*), USA, North Dakota, Bismarck, 30.VIII.1884, A.B. Seymour, BPI 69513; isotypes HUV 1980, Ellis & Ev., N. Amer. fgi. no. 2265/a, HUV 12914!

Sori in all sessile and pedicelled spikelets of an inflorescence, cylindrical, 1-1.5 x 5-10 mm, initially covered by a thick, light brown, fungal peridium which ruptures irregularly at maturity disclosing the blackish-brown, semiagglutinated mass of spore balls, spores and sterile cells surrounding a simple, narrowing, central columella. *Spore balls* loose, early disintegrating. *Spores* slightly irregular, subpolyhedral, ovoid to oblong, 12-16 x 13.5-18 µm, yellowish-brown; wall uniform, thin, c. 0.5 µm, finely and densely punctate-echinulate, spore profile finely serrulate. *Spore germination* (on MYP, at room temp., in 4 days) results in 4-celled basidia the cells of which give rise to long, septate, ramifying hyphae. *Sterile cells* in groups or chains, very variable in shape and size, usually smaller than the spores, 8-15 µm long, rarely up to 22 µm long, collapsed in old specimens, hyaline, with a few oil droplets; wall thin, c. 0.5 µm, smooth.

On *Andropogon gerardii* Vitman (*A. furcatus* Muhl.; *A. provincialis* Lam. not Retz.), *A. glomeratus* (Walter) Britton, Stern. & Poggenb., *A. hallii* Hack., *A. virginicus* L., *Schizachyrium scoparium* (Michx.) Nash (*Andropogon scoparius* Michx.); N. America (Canada, USA).

24. ***Sporisorium polliniae*** (Magnus) Vánky, 1983:331.

Sorosporium polliniae Magnus, 1900:433 (as '*Sorisporium*'). — Type on *Pollinia distachya* (= *Andropogon distachyos*), Judaea [= Israel], Jaffa Distr., Bab-el-Wad, 15.V.1897, J. Bornmüller 1015; isotypes in Bornmüller, Iter syriacum 1897, no. 1015, BPI 180134!

Sorosporium icosiense Maire, 1917:145. — Type on *Andropogon distachyos*, Algeria, near Icosium, Télemly, El-Biar, coll. R. Maire. (syn. by Ling, 1951:47).

Sori in all, both sessile and pedicelled spikelets of an inflorescence, destroying the innermost floral organs, cylindrical or corniculate, 1-1.5 x 7-10 mm, partly hidden by the glumes, covered by a yellowish-brown peridium which splits longitudinally in several places exposing the blackish-brown, granular-powdery mass of spore balls and a central, filiform, sometimes apically bifurcate columella. *Spore balls* subglobose, ellipsoidal to irregular, 20-40 x 25-55 µm, reddish-brown, composed of few to many (10-40 or more?) spores, initially firm but disintegrating readily at maturity. *Spores* subglobose, ellipsoidal, usually slightly subpolyhedrally irregular, 7-11 x 8-14 µm, yellowish- to reddish-brown, verrucose to echinulate on the free surface, punctate to apparently smooth on the contact sides; wall uneven, 1-2 µm thick, thickest at the angles and on the free surface, inner spores pale yellowish-brown, wall even, c. 0.5 µm thick, smooth. *Spore germination* (on potatoglucoseagar, at room temp., in 2 days) results in long, 4-celled basidia with fusiform basidiospores on short sterigmata at the septa and on the apex of the basidium. *Sterile cells* absent. The peridium is composed of long chains of hyaline, thin-walled, elongated or irregular cells (8-16(-20) µm long), with 1(-2) oil droplets.

On *Andropogon abyssinicus* Fresen., *A. distachyos* L. (*Pollinia distachya* (L.) Spreng.; *Chrysopogon distachyos* (L.) Rossi); Mediterranean region (S. Europe, N. & NE. Africa, incl. Ethiopia, Asia).

Specimens collected in Ethiopia have larger and more compact spore balls. In other respects they are identical with the type.

25. ***Sporisorium provinciale*** (Ellis & Galloway) Vánky & Snets., in Vánky, 1990:271.

Sorosporium ellisii var. *provinciale* Ellis & Galloway, 1890:31. — *Sorosporium provinciale* (Ellis & Galloway) G.P. Clinton, 1902:145. — Lectotype on *Andropogon provincialis* (= *A. gerardii*), USA, (design. by Clinton, 1902:145) Montana, Saline Co., near Emma, 3.VI.1889, C.H. Demetrio, BPI 179762; isolectotypes in Ellis & Ev., N. Amer. fgi. no. 2425, HUV 5231! Syntype BPI 179759.

Sori on the top of shoots comprising the whole inflorescence and inflorescence axis, cylindrical, 2-5 mm wide, up to 12 cm long, sometimes bearing remnants of inflorescence branches or spikelets distally, partly enclosed by the leaf sheath, first covered by a light brown peridium which lacerates at maturity disclosing the blackish-brown, granular-powdery mass of spore balls and the interwoven and interconnected, filiform columellae of remnants of host vascular tissue. *Spore balls* globoid to oblong or somewhat irregular, 25-55 x 30-75 µm, medium dark yellowish-brown, composed of 5-35 (or more?), loosely connected spores. *Spores* rather uniform, globose, ovoid or slightly irregularly subpolyhedral, 12-16 x 13-19 µm, yellowish-brown; wall 3-4 µm thick, minutely, sparsely to moderately densely verrucose, spore profile finely wavy. *Spore germination* (on MYP, at room temp., in one week) results in 4-celled basidia on which long, cylindrical or naviculiform basidiospores are produced on well developed sterigmata. The basidiospores become multiseptate and produce small, slightly bent secondary sporidia. *Sterile cells* in groups or chains, single cells varying in shape and size, usually smaller than the spores, 8-15, rarely up to 22 μm long, collapsed in old specimens, hyaline, with a few oil droplets; wall thin, c. 0.5 μm, smooth.

On *Andropogon gerardii* Vitm an (*A. provincialis* Lam.; *A. furcatus* Muhl.), *A. hallii* Hack., *A. microstachyus* Desv., *A. virginicus* L., *Schizachyrium hirtiflorum* Nees (*Andropogon hirtiflorus* (Nees) Kunth), *S. scoparium* (Michx.) Nash (*Andropogon scoparius* Michx.); N. America (Mexico, USA).

26. *Sporisorium pseudomaranguense* (Zundel) Vánky, 2005a:262.

Sorosporium pseudomaranguense Zundel, 1938:309. - Type on *Andropogon* sp. (det. A. Chase), South Africa, Natal, Mooi River, 21.III.1917, A.O.D. Mogg, PREM 10073; isotype HUV 18007!

Sori destroying the whole inflorescence, long linear, c. 2 mm wide, 3-5 cm long, hidden by the uppermost leaf sheath from which only the tips of the sori protrude, initially covered by a thick, yellowish-brown peridium which ruptures irregularly liberating the dark brown, granular-powdery mass of spore balls and groups of sterile cells surrounding numerous, long, filiform, slender columellae. *Spore balls* subglobose, ovoid, ellipsoidal, oblong or slightly irregular, 30-70 x 40-130 μm, dark reddish-brown to subopaque, composed of tens of spores that separate by pressure. *Spores* varying in shape and size, subglobose, ellipsoidal to subpolyhedrally irregular, 8-12 x 9-14.5 μm, dimorphic. Outer spores rounded, larger, dark yellowish- to reddish-brown; wall irregular, 0.8-1.2 μm thick, prominently, moderately densely verrucose-echinulate, spore profile serrulate. Inner spores subpolyhedral, smaller, pale yellowish-brown; wall even, c. 0.5 μm thick, finely punctate-verruculose. *Sterile cells* few, in irregular groups or short chains, single cells ellipsoidal to usually irregular with flattened sides, 8-12 μm long, hyaline; wall c. 0.5 μm thick, smooth.

On *Andropogon* sp.; S. Africa. Known only from the type collection.

27. *Sporisorium sanctae-catharinae* (Zundel) Vánky, **comb. nov.**

Basionym: *Ustilago sanctae-catharinae* Zundel, Mycologia 43:268, 1951 (nom. nov.). — *Ustilago occulta* Hennings, 1897:212 (later homonym, not *U. occulta* (Wallr.) Rabenh., Herb. viv. myc. no. 1898, 1874). — Type on *Andropogon* sp., Brazil, Santa Catarina, Caraça, III.1892, E. Ule 1888, HBG!

Sori on the top of sterile shoots including also the uppermost leaves and leaf sheaths, transforming them into slender, bent, cylindrical, distally narrowing bodies, up to 5 mm wide and 10 cm long, initially covered by host epidermis which ruptures longitudinally in many places disclosing the dark brown, semiagglutinated to powdery mass of spores, sterile cells and numerous filiform columellae of vascular bundles. *Spores* globose, subglobose, broadly ellipsoidal, 5.5-7(-8) x 6.5-9 μm, dark yellowish-brown; wall 1-1.5 μm thick including the cylindrical, often anastomosing, labyrinthiform warts, with flattened tip; in SEM the spore surface appears cerebriform. *Sterile cells* in small groups, single cells subglobose, ellipsoidal or slightly irregular, 10-15 μm long, yellowish-brown, collapsed in old specimen; wall even, c. 0.5 μm thick, finely punctate-echinulate, profile smooth to finely wavy.

On *Andropogon* sp.; S. America (Brazil). Known only from the type locality.

Sporisorium sanctae-catharinae is closest to *S. mexicanum* (type on *Andropogon gerardii*), from which it differs by smaller spores and lower warts with flattened tip.

28. *Sporisorium scholzii* Vánky, sp. nov.

Typus in matrice Andropogon schirensis *A. Rich., Zambia, Eastern Prov., 407 km ENE urbe Lusaka, 14°16'54" S, 31°37'41" E, alt. 1070 m.s.m., 18.IV.2001, leg. C., T. & K. Vánky. Holotypus in Herbario Ustil. Vánky, HUV 21062! Paratypus in matrice* Andropogon schirensis, *Zambia, Lusaka Prov., 124 km ENE urbe Lusaka, 15°08'59" S, 29°21'45" E, alt. 1035 m.s.m., 17.IV.2001, leg. T., C. & K. Vánky, HUV 21065!*

Sori *in spiculis nonnullis sessilibus inflorescentiae eiusdem, longe cylindrici, 1-1,5 x 7-15 mm, primo peridio crasso, cremeo cooperti, quo apice eius irregulariter rupto massam nigram, granuloso-pulveream glomerulorum sporarum et columellarum nonnullarum, longarum filiformium ostendentes.* Glomeruli sporarum *forma et magnitudine varii, subpolyedrice irregulares, interdum elongati, raro globosi, 70-140 x 80-180(-200) µm, atro-rubrobrunnei usque plerumque opaci, permanentes, e sporis pluries decem vel pluries centum pressu valido tantum difficiliter separatis compositi.* Sporae *dimorphae, externae earum globosae, subglobosae, ellipsoidales usque rotundato-subpolyedricae, 9-10,5 x 9-11(-12) µm, mediocriter atro-olivaceobrunneae; pariete inaequali, 0,5-1 µm crasso, in superficie libera valde leniter punctato-verruculoso, imago obliqua sporarum levis. Sporae internae rotundate subpolyedricae vel polyedricae, magnitudine sporis externis cca. aequales, pallide olivaceobrunneae; pariete aequali, 0,3-0,5 µm crasso, levi, sub SEM ruguloso.* Cellulae steriles *absentes.*

Sori (Fig. 12) in some sessile spikelets of an inflorescence, long cylindrical, 1-1.5 x 7-15 mm, initially covered by a thick, cream coloured peridium which ruptures irregularly from its apex disclosing the black, granular-powdery mass of spore balls and several long, filiform, columellae. *Spore balls* (Figs. 17, 18) varying in shape and size, subpolyhedrally irregular, sometimes elongated, rarely globose, 70-140 x 80-180(-200) µm, dark reddish-brown to usually opaque, permanent, composed of tens or hundreds of spores which separate with difficultly by hard pressure. *Spores* (Figs. 17, 18) dimorphic, outer spores globose, subglobose, ellipsoidal to rounded subpolyhedral, 9-10.5 x 9-11(-12) µm, medium dark olivaceous-brown; wall uneven, 0.5-1 µm thick, finely punctate-verruculose on the free surface, spore profile smooth. Inner spores rounded subpolyhedral or polyhedral, about the size of the outer spores, pale olivaceous-brown; wall even, 0.3-0.5 µm thick, smooth, in SEM appearing wrinkled. *Sterile cells* absent.

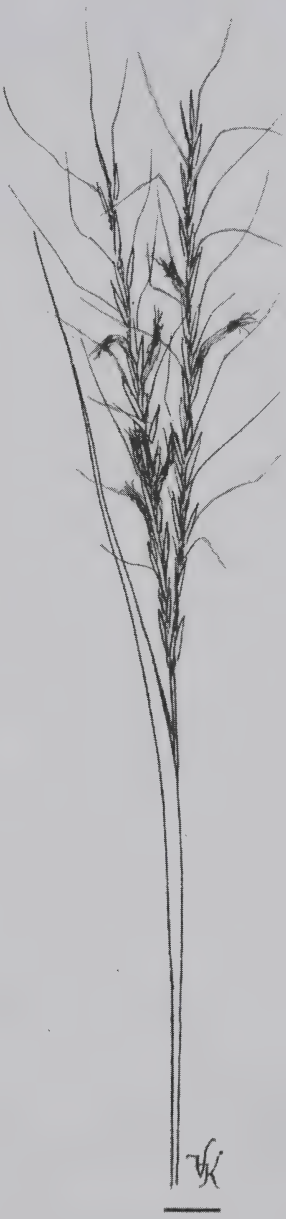
On *Andropogon schirensis* A. Rich.; C. Africa. Known only from the type collections.

Sporisorium scholzii differs from *S. andropogonis-pumili* in which the spore balls are larger and darker, and the outer spores of the balls are provided with densely situated, 1-2.5 µm long, filiform warts. It differs also from *S. livingstoneanum* in which the columellae are often band-like, the spore balls are smaller, and the outer spores of the balls are provided with moderately densely situated, 0.5(-1) µm high warts or spines.

Etymology: This species is named in the honour of Professor Hildemar Scholz (Berlin, Germany), an excellent and helpful person, outstanding specialist of the *Poaceae* of the world, and also of the smut fungi, author of several books on grasses and on the smut fungi of Germany (1988a, b, 2000, 2004).

29. *Sporisorium seymourianum* (G.P. Clinton) M. Piepenbring, 2003:89.

Sphacelotheca seymouriana G.P. Clinton, 1904:387. — Lectotype on *Andropogon virginicus*, USA, Alabama, Auburn, 14.X.1897, Earle & Baker, (design. by Piepenbring, 2003:89) BPI 193990; isolectotypes BPI 193989, Seymour & Earle, Econ. Fgi., Suppl. C. no. 531, HUV 17711!, Syd. Ust. no. 189, HUV 1660! Syntypes in Seymour & Earle, Econ. Fgi., Suppl. C. no. 122, HUV 9710!



12

Fig. 12. Sori of *Sporisorium scholzii* in some sessile spikelets of *Andropogon schirensis* (type). Habit. Bar = 1 cm.

Sori in all sessile and pedicelled spikelets of an inflorescence, cylindrical, 0.5-1 x 3-7 mm, partly hidden by floral envelopes, first covered by a pale brown peridium which ruptures from its apex disclosing the dark brown, agglutinated to powdery mass of spore balls, spores and sterile cells surrounding a simple, narrowing, central columella. *Spore balls* irregular, early disintegrating. *Spores* rounded, subpolyhedrally slightly irregular, 7-11 x 8-13.5 µm, yellowish-brown; wall even, c. 0.5 µm thick, finely, densely punctate-verruculose, spore profile smooth. *Sterile cells* in irregular groups, single cells about the size of the spores, hyaline, collapsed in old specimens; wall c. 0.5 µm thick, smooth.

On *Andropogon gerardii* Vitman, *A. ternarius* Michx., *A. virginicus* L., *Schizachyrium scoparium* (Michx.) Nash (*Andropogon scoparius* Michx.); N. America (USA).

30. ***Sporisorium stuhlmannii*** (Henn.) Vánky, 2004a:107.

Ustilago stuhlmannii Hennings, 1893:3. — *Sphacelotheca stuhlmannii* (Henn.) Zundel, 1930:136. — Type on *Andropogon* sp., "Central Afrikan. Seengebiet" (Central African Lakes Territory), Ukami, Mrogoro, 18.V.1890, F. Stuhlmann 63, "Emin Pascha Expedition", BPI 198126!

Ustilago andropogonis-hirtifolii Henn., in Holway, 1899:274 (as '*andropogonis-hirtefolii*'). — *Sphacelotheca andropogonis-hirtifolii* (Henn.) G.P. Clinton, 1902:141. — *Sporisorium andropogonis-hirtifolii* (Henn.) M. Piepenbring, 2003:88. — Type on *Andropogon hirtifolius* var. *pubiflorus*, Mexico, Michoacán, Patzcuaro, 20.X.1898, E.W.D. Holway 3216, B; isotypes BPI 157091, 157093, HBG, Sydow, Ust. no. 201, HUV 9766! (syn. nov.)

Ustilago andropogonis-saccharoidis Henn., in P. Sydow, Ustilagineen no. 251 (nom. nud.). — *Sphacelotheca andropogonis-saccharoidis* (Henn.) Cif., in Ciferri & Herter, 1932:531 (comb. invalid.). — Type on *Andropogon saccharoides*, Mexico, Jalisco, Chapala, 17.IX.1899, E.W.D. Holway; isotypes in Syd. Ustil. no. 251, HUV 3154! (syn. by Clinton, 1902:129). The host plant in the HUV copy of Syd. Ustil. no. 251 is *Andropogon perforatus* (det. K. Vánky).

Sori comprising the entire inflorescence, elongate, 1-3 mm wide, 5-10 cm long, partly enclosed by leaf sheaths, initially covered by a thick, brown peridium which flakes away disclosing the semiagglutinated to powdery, dark olivaceous-brown mass of spores and sterile cells surrounding a long, sometimes bifurcate columella often with short lateral branches. Sori rarely restricted to the spikelets, then c. 1 x 5-10 mm. *Spores* subglobose, ellipsoidal to usually rounded, subpolyhedrally slightly irregular, 8-12 x 9-13.5 µm, yellowish-brown; wall even, c. 0.5 µm thick, finely, densely punctate to verruculose-echinulate, spore profile smooth, finely wavy to finely serrulate. *Spore germination* (Durán, 1987:87 & 102) results in 4-5-celled basidia producing basidiospores. *Sterile cells* in irregular groups, single cells 6.5-13 µm long, subhyaline; wall thin, c. 0.5 µm, smooth, collapsed in old specimens.

On *Andropogon barbinodis* Lag. (*Bothriochloa barbinodis* (Lag.) Herter), *A. hallii* Hack., *A. hirtifolius* J. Presl var. *pubiflorus* Hack., *A. lateralis* Nees, *A. perforatus* Trin. ex Fourn., *A. saccharoides* Sw. (*Bothriochloa saccharoides* (Sw.) Rydb.), *A. wrightii* Hack., *Andropogon* sp.; Africa, N. America (Mexico, USA), Caribic Islands (Dominican Rep.).

The sori, spores and sterile cells of the types of *Ustilago andropogonis-hirtifolii* and those of *U. stuhlmannii* are identical hence they are considered to be synonyms.

31. ***Sporisorium zilligii*** (Zundel) Vánky, **comb. nov.**

Basionym: *Sphacelotheca zilligii* Zundel, Mycologia 22:142, 1930. — Type on *Andropogon* sp., South Africa, Cape Province, Vryburg, 25.III.1921, A.O.D. Mogg, PREM 20666; isotypes BPI 192099, BPI 195088, HUV 18128!

Sori destroying the whole inflorescence, long linear, 1-2 x 10-30 mm, partly hidden by leaf sheaths, initially covered by a thick, brown peridium which flakes away disclosing the dark brown, semiagglutinated to powdery mass of spore balls, spores and sterile cells surrounding a stout, bifurcate or much ramifying columella. *Spore balls* loose, composed of tens or a hundred of easily separating spores. *Spores* globose, ovoid, ellipsoidal to slightly irregular, varying in size, 5.5-9.5(-10.5) x 6-13(-16) µm, yellowish-brown; wall even, c. 0.5 µm thick, finely, densely punctate-verruculose, spore profile smooth. *Sterile cells* in irregular groups, single cells subglobose, ellipsoidal, irregular, 8-16(-18) µm long, subhyaline to pale yellowish-brown tinted, collapsed in old specimen; wall c. 1 µm thick, smooth.

On *Andropogon* sp.; S. Africa. Known only from the type collection.

Doidge (1950:384), and after her Zundel (1953:115), stated that the host plant is a *Cymbopogon* sp., not *Andropogon* as originally given. Unfortunately, no healthy host plant is preserved. Judged from the sori, I doubt that the host is a *Cymbopogon* (comp. Vánky, 2003:31).

32. ***Ustilago andropogonis-tuberculati*** Brefeld, 1895:108.

Type on *Andropogon tuberculatus*, India, Simla, comm. Barclay (type where?).

Sori in the ovaries, compact, hard, black. *Spores* remarkably variable, rounded, 10-13 µm in diameter, dark brown. *Spore germination* (Brefeld, 1895:108, Plate VI, figs. 24, 25) results in long, narrow, 4-celled basidia on which long ellipsoidal basidiospores are produced.

On *Andropogon tuberculatus* Hack.; S. Asia (India).

No specimen was available for study. Description taken from the original. Collection and study of this species is desired as the generic position is uncertain.

Invalid names, uncertain, excluded and undescribed species

Sphacelotheca furcata var. ***congoensis*** Zambettakis, 1979(1980):410.

Invalid name, no Latin diagnosis, and no type designated (ICBN 36.1 & 37.1). On *Andropogon* sp., Congo, BR.

Sphacelotheca tonkinensis (Henn.) Zundel, 1930:134.

Uredo tonkinensis Hennings, 1895a:11. — Type on *Andropogon* sp., N. Vietnam, Tonkin, Hanoi (type where?).

Sori destroying the ovaries, 2-3 mm long, at first concealed by the glumes, covered by a brown peridium, which dehisces from the apex revealing a brown spore mass surrounding a well-developed columella. *Spores* globose to subglobose, regular, 9-12 µm in diameter, reddish-brown; wall thick, two-layered, smooth. Spore content granular-vacuolate.

Type not seen. Description taken from Zundel (1930:134). This species belongs to the genus *Sporisorium*. However, the short and incomplete description does not allow a sure identification.

Sporisorium moniliferum (Ellis & Everh.) L. Guo, 1990:82.

Type on *Heteropogon contortus* (L.) P. Beauv. ex Roem. & Schult., USA.

Zundel (1953:100) mentions this smut also on *Andropogon glomeratus* (Walter) Britton, Stern. & Poggenb. from the USA, Va. The specimen in BPI 178090! (USA, Va., Williamsburg, 28.VI.1921, E.J. Grimes) represents another, probably an undescribed species.

Ustilago amadelpha is *Ustilago scitaminea*

Ustilago amadelpha Syd., P. Syd. & E.J. Butler, 1912:249. — Type on "*Andropogon* sp." (= misnamed *Saccharum* sp.), India.

It is identical with the later published *Ustilago scitaminea* Syd. on *Saccharum officinarum* L., India (Vánky, 2004a:114), a name that was proposed for conservation (Vánky & Shivas, 2005:180).

Sporisorium sp. nov.

On *Andropogon gayanus* Kunth, Nigeria, Prov. Zaria, Samaru, 20.II.1961, E. Harris, IMI 87111!

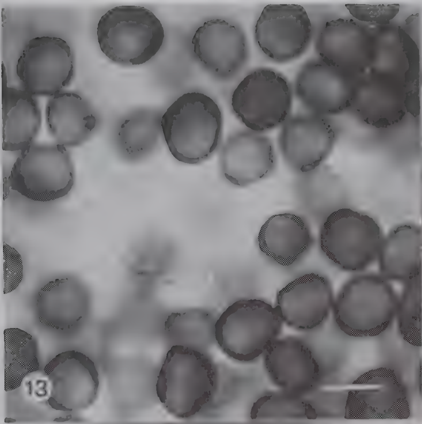
Sori in the spikelets, cylindrical, up to 2 cm long, covered by a peridium which ruptures disclosing the dark brown, granular powdery mass of spore balls surrounding a central columella. *Spore balls* variable in shape and size, ovoid, usually oblong or irregular, 40-120 x 50-180(-210) µm, olivaceous brown, rather permanent, composed of tens to hundreds of spores. *Spores* dimorphic, outer spores ellipsoidal, 6.5-9 x 6.5-10(-11) µm, brown; wall c. 1 µm thick, on the free surface prominently verrucose. Inner spores subpolyhedrally irregular, subhyaline with c. 0.5 µm thick, smooth wall. *Sterile cells* not seen

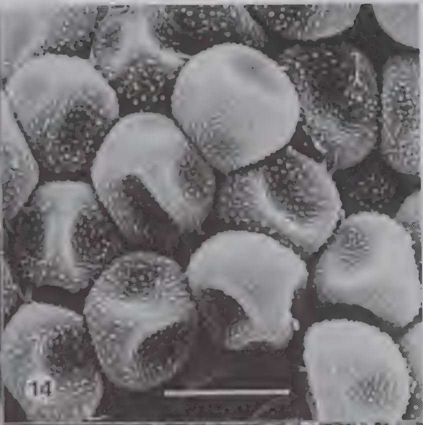
It differs from all known *Sporisorium* species on *Andropogon*. However, the IMI specimen I have seen is too scanty to be designated as the type of a new species. It is presented here to call attention to it.

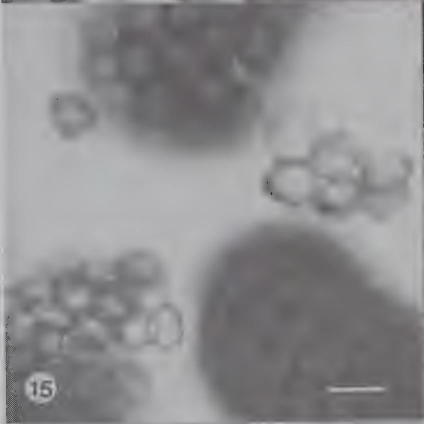
Figs. 13, 14. Spores and sterile cells of *Sporisorium distachyum* on *Andropogon distachyos*, in LM and in SEM (type).

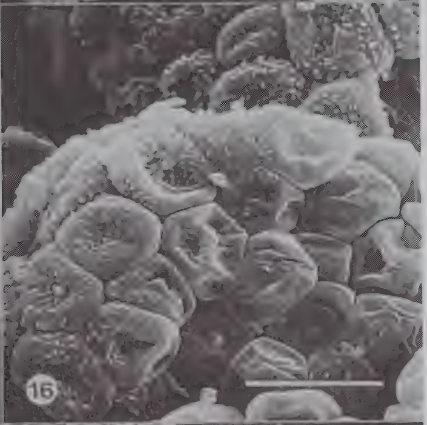
Figs. 15, 16. Spore balls and spores of *Sporisorium livingstoneanum* on *Andropogon gayanus*, in LM and in SEM (type).

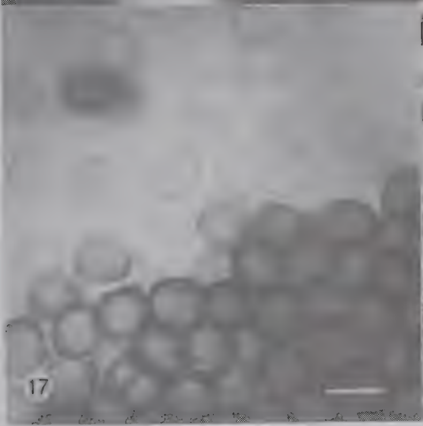
Figs. 17, 18. Spore balls and spores of *Sporisorium scholzii* on *Andropogon schirensis*, in LM and in SEM (type). Bars = 10 µm.

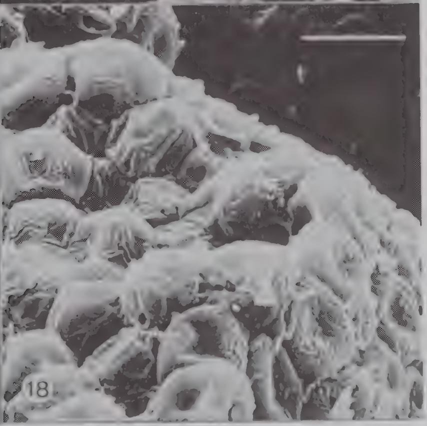

13


14


15


16


17


18

Key to the smut fungi of *Andropogon* and *Schizachyrium*

(S. = Sporisorium)

1. Sori on the leaves or leaf sheaths as striae. Spore wall thick, multilayered 2
— Sori and spore wall not so 3
2. Spores 10-20(-24) µm long; wall 3-8 µm thick .... ***Jamesdicksonia brunkii***
— Spores 8-16(-18) µm long; wall 1.5-6.5 µm thick .... ***Jamesdicksonia caribensis***
3. Sori on the top of shoots as whip-like structures 4
— Sori not so 7
4. Columella one, stout, with longitudinal furrows. Spore wall 0.4-0.8 µm thick, thinner on one side .... ***S. andropogonis-tectorum***
— Columellae several, filiform. Spore wall thicker, not thinner on one side 5
5. Spores 13-19 µm long; wall 3-4 µm thick, minutely verruculose .... ***S. provinciale***
— Spores smaller; wall thinner 6
6. Spores 8-11 µm long; wall 2-2.5 µm thick, including the 1.5-2 µm high, anastomosing warts .... ***S. mexicanum***
— Spores 6.5-9 µm long; wall 1-1.5 µm thick, including the cylindrical, single or anastomosing, labyrinthiform or cerebriform warts .... ***S. sanctae-catharinae***
7(3). Sori destroying the whole inflorescence 8
— Sori restricted to the spikelets or ovaries 19
8. Spores dimorphic. Columella filiform, one or numerous. 9
— Spores not dimorphic. Columella stout, one, sometimes branching 14
9. Columella one 10
— Columellae numerous 13
10. Spore balls persistent 11
— Spore balls easily separating 12
11. Spores 10.5-13.5 µm long .... ***S. absconditum***
— Spores 15-20(-21.5) µm long .... ***S. blakeanum***
12. Spores 10.5-17 µm long, free surface coarsely echinulate .... ***S. zambianum***
— Spores 13-20 µm long, free surface finely verrucose .... ***S. guaraniticum***
13. Spores 13.5-18.5(-20) µm long. Sterile cells absent .... ***S. andropogonis-eucomi***
— Spores 9-14.5 µm long. Sterile cells present .... ***S. pseudomaranguense***
14. Spores very variable in size, 6-13(-16) µm long .... ***S. zilligii***
— Spores less variable in size 15
15. Spores up to 20 µm long 16
— Spores smaller 17
16. Spores regular, with a rounded paler area .... ***S. culmiperdum***
— Spores mostly slightly irregular, with irregular, paler and darker areas .... ***S. ellisii***
17. Spores 7.5-11 µm long .... ***S. andropogonis***
— Spores larger 18
18. Spores 9-13.5 µm long; wall even, 0.5 µm thick .... ***S. stuhlmannii***
— Spores 12-16 µm long; wall uneven, 0.5-1.5 µm thick .... ***S. leucostachys***
19(7). Sori in the ovaries, compact, hard, black .... ***Ustilago andropogonis-tuberculati***
— Sori in the spikelets, not hard and black 20

20. Sori in some spikelets of an inflorescence 21
— Sori in all spikelets, both sessile and pedicelled, or only sessile ones of an inflorescence (excepting sometimes *S. everhartii*) 27
21. Sori producing groups of witches' brooms in the inflorescence 22
— Sori not producing witches‘ brooms 23
22. Spores 10-16(-18) μm long .... ***S. holwayi***
— Spores 6.5-11(-12) μm long .... ***S. bicornis***
23. Spores not in permanent balls, not dimorphic 24
— Spores in permanent balls, dimorphic 25
24. Spores prominently echinulate, single .... ***Macalpinomyces ovariicolopsis***
— Spores finely punctate-verruculose, in loose balls .... ***S. berndtii***
25. Columella one, simple. Warts 1-2.5 μm long, filiform .... ***S. andropogonis-pumili***
— Columellae several, or one, fused, band-like. Warts shorter, not filiform 26
26. Columellae several. Outer spores finely punctate-verruculose .... ***S. scholzii***
— Columella often band-like. Outer spores with 0.5(-1) μm high warts or spines .... ***S. livingstoneanum***
27(20). Columellae several, filiform (or one, filiform in *S. schizachyrii*) 28
— Columella one, flagelliform or stout, simple or with branches 30
28. Spores not dimorphic, 7-10 μm long .... ***S. andropogonis-gabonensis***
— Spores dimorphic, larger 29
29. Spores 8-10.5 μm long. Sterile cells absent .... ***S. schizachyrii***
— Spores 10.5-14.5 μm long. Sterile cells present .... ***S. gayanum***
30. Spores dimorphic 31
— Spores not dimorphic. Spore balls ephemeral or very loose 33
31. Spore balls loose, 25-40(-50) μm long .... ***S. andropogonis-chinensis***
— Spore balls permanent, longer 32
32. Spore balls 50-130 μm long. Outer spores 8-15 μm long .... ***S. everhartii***
— Spore balls 50-180(-210) μm long. Outer spores 6.5-10 μm long .... ***S. sp.***
33. Spores 13.5-18 μm long .... ***S. occidentale***
— Spores smaller 34
34. Sterile cells absent 35
— Sterile cells present 36
35. Spore wall 0.5-1.2 μm thick, thinner on one side .... ***S. braziliense***
— Spore wall 1-2 μm thick, uneven, thickest at the angles .... ***S. polliniae***
35. Spores 7-10.5(-11) μm long .... ***S. andropogonis-schirensis***
— Spores larger, up to 13.5 μm long 37
37. Spore wall 0.5-1(-1.5) μm thick, with a paler, thinner germ pore, finely, densely verrucose-echinulate .... ***S. distachyum***
— Spore wall thinner, germ pore lacking, more finely ornamented 38
38. Sori completely destroying the spikelets. Spore wall 0.5-0.8 μm thick. Sterile cells in compact, rounded or elongated groups .... ***S. fastigiatum***
— Sori not so. Spore wall c. 0.5 μm thick. Sterile cells not so .... ***S. seymourianum***

The smut fungi of *Sorghastrum* (*Poaceae*)

Sorghastrum Nash, in the subfam. *Panicoideae*, tribe *Andropogoneae*, subtribe *Sorghinae*, is a genus of about 16 species in Africa and tropical America. It is a relative of *Sorghum* (Clayton & Renvoize, 1986:341). Five smut fungi could be recognised on *Sorghastrum:*

1. ***Macalpinomyces ugandensis*** Vánky, 2003:50.

Type on *Sorghastrum stipoides*, Uganda, Rakai Distr., 40 km SW. of Masaka, 6 km NE. of Kyotera, alt. 1190 m, 27.II.2002, T., C. & K. Vánky, HUV 19992; isotypes BPI, MHU, Vánky, Ust. exs. no. 1177. Paratype on *Loudetia phragmitoides*, HUV 19993; isoparatypes BPI, K, MHU.

For description and illustrations see Vánky, 2003:50-52 & 55.

On *Sorghastrum stipoides* (Kunth) Nash, and *Loudetia phragmitoides* (Peter) C.E. Hubb.; C. Africa (Uganda).

2. ***Sporisorium clintonianum*** Vánky, **nom. nov.**

Replacing *Sphacelotheca chrysopogonis* G.P. Clinton, Proc. Boston Soc. Nat. Hist. 31:387, 1904 (not *Sporisorium chrysopogonis* Vánky, 1983:327). — Type on *Chrysopogon nutans* (= *Sorghastrum nutans*), Mexico, Chapala, 1901, E.W.D. Holway, BPI 177307; isotopotypes in Seymour & Earle, Econ. fgi., Suppl. C, no. 118, HUV 9706!

Sori in all ovaries of an inflorescence, c. 3-5 mm long, showing between the spreading floral envelopes, initially covered by a thin peridium which ruptures disclosing the blackish-brown, semiagglutinated to powdery mass of spores and sterile cells, surrounding a simple, central columella. *Spores* single when mature, subglobose, ellipsoidal to usually slightly irregular, 7-9.5 x 7.5-10.5 µm, light yellowish-brown; wall even, c. 0.5 µm thick, finely, densely punctate, spore profile smooth. *Sterile cells* in large, irregular groups, single cells globoid, ellipsoidal or irregular, with flattened contact sides, 6.5-12 x 6.5-13 µm, subhyaline; wall c. 1 µm thick, smooth, collapsed in old specimens.

On *Sorghastrum nutans* (L.) Nash (*Chrysopogon nutans* (L.) Benth.); N. America (Mexico, USA).

Fischer (1953:134) considered *Sphacelotheca chrysopogonis* to be a synonym of *S. cruenta* (J.G. Kühn) Potter (= *Sporisorium cruentum* (J.G. Kühn.) Vánky, type on *Sorghum saccharatum* (L.) Pers.). However, the two differ in sorus, spore and sterile cell morphology which, in my opinion, is sufficient to treat them as two separate species.

3. ***Sporisorium sorghastri*** (Zundel) Vánky, **comb. nov.**

Basionym: *Sphacelotheca sorghastri* Zundel, in Massey & Zundel, Phytopathology 32:545, 1942. — Type on *Sorghastrum elliottii*, USA, Virginia, Pittsylvania Co., 2 miles W of Chatham, Moses Mill Pond, 4.IX.1941, A.B. Massey 5059, BPI 193999!

Sori destroying the whole inflorescence, long linear, 2-4 mm wide, 7-10 cm long, at first covered by a thick, pale brown peridium that ruptures irregularly, flakes away, exposing the dark brown, semiagglutinated to powdery mass of spores and sterile cells surrounding a central columella with several long, filiform branches. *Spores* single when mature, subglobose, ovoid, ellipsoidal to slightly irregular, 7-10(-11) x 8-11(-12) µm, yellowish-brown; wall even, c. 0.5 µm thick, finely, densely punctate-verruculose, spore profile smooth. *Sterile cells* in irregular groups or chains, single cells ellipsoidal or irregular, with flattened contact sides, 7-12 x 8-16 µm, hyaline; wall thin, c. 0.5 µm, smooth, collapsed in old specimens.

On *Sorghastrum elliottii* (Mohr) Nash; N. America (USA).

4. *Sporisorium tepicense* (Durán) M. Piepenbring, 2003:129.

Sphacelotheca tepicensis Durán, 1970:1098. — Type on *Sorghastrum incompletum*, Mexico, Nayarit State, 38.6 km SE of Tepic, Hwy. Mex. no. 15, 29.X.1969, R. Durán, WSP 58550; isotype HUV 14419!

Sori in all ovaries of an inflorescence, ovoid or ellipsoidal, 0.5-0.8 x 1-2 mm, showing between the spreading floral envelopes, first covered by a thin, fragile, dark brown peridium which ruptures irregularly at maturity disclosing the blackish-brown, semiagglutinated mass of spores and sterile cells surrounding a thin, central columella of the length of the sorus. *Spores* single when mature, globose, ovoid or ellipsoidal, 6.5-9 x 7-11 µm, medium dark reddish-brown; wall even, 0.5-1 µm thick, densely, coarsely verrucose, spore profile wavy to finely serrulate. *Sterile cells* in short chains, or in small groups, single cells globoid, ellipsoidal to usually irregular with flattened contact sides, 5.5-12 x 5.5-15 µm, pale yellowish-brown; wall c. 1 µm thick, smooth.

On *Sorghastrum incompletum* (J. Presl) Nash; N. America (Mexico). Known only from the type collection.

5. *Tolyposporella chrysopogonis* G.F. Atkinson, 1897:16.

Type on *Chrysopogon nutans* (= *Sorghastrum nutans*), USA, Alabama, Auburn, autumn, Duggar, no special collection designated.

Sori on inner surface of leaf sheaths, by which they are concealed, forming linear, more or less confluent, initially subepidermal, later bursting striae filled with black, granular-agglutinated masses of spore balls. *Spore balls* subglobose, oblong, subpolyhedral, usually more or less irregular, 40-80 x 50-175 µm, reddish- to blackish-brown, opaque, composed of numerous, firmly agglutinated spores, separating with difficulty. *Spores* subglobose, ovoid, usually irregular, (7-)8-9.5 x 8-13(-15) µm including the thin (c. 1 µm), dark endospore but excluding the irregularly thickened (2.5-8(-14) µm), laminated, light yellowish-brown, smooth exospore which, on the outer spores of the ball may be excessively thickened forming long (up to 14 µm), often bent appendages; with such thickenings of the spore wall the spores may reach 30 µm in length. Spores "germinating by a delicate promycelium which becomes branched, septate. Sporidia borne laterally, single, subclavate or fusoid, 2-2.5 x 9-12 µm" (Atkinson, 1897:16).

On *Sorghastrum nutans* (L.) Nash (*Chrysopogon nutans* (L.) Benth.; *C. avenaceus* (Michx.) Benth.; *S. avenaceum* (Michx.) Nash), *S. stipoides* (Kunth) Nash; C. Africa (Uganda), N. America (USA).

Key to the smut fungi of *Sorghastrum*

1. Sori on the inner surface of leaf sheaths. Spore wall 3-9(-15) µm thick .... ***Tolyposporella chrysopogonis***
– Sori elsewhere. Spore wall 0.5-1 µm thick 2
2. Sori on the top of shoots forming up to 1 m long, flagelliform tubes .... ***Macalpinomyces ugandensis***
– Sori not so 3
3. Sori in the whole inflorescence .... ***Sporisorium sorghastri***
– Sori in the ovaries 4
4. Spores densely, coarsely verrucose .... ***Sporisorium tepicense***
– Spores densely, finely punctate .... ***Sporisorium clintonianum***

The smut fungi of *Leptochloa* (*Poaceae*)

Leptochloa P. Beauv. (including *Diplachne* P. Beauv.), in the subfam. *Chloridoideae*, tribe *Eragrostideae*, subtribe *Eleusinae*, is a genus of 40 species throughout the tropics, warm temperate parts of America and Australia (Clayton & Renvoize, 1986:208). On *Leptochloa* and *Diplachne* seven smut fungi are recognised:

1. *Tilletia leptochloae* Thirumalachar & Pavgi, 1968(1969):253.

Type on *Leptochloa filiformis* (= *L. panicea*), India, Rajasthan, Ajmer, X.1955, P.N. Mathur 1259, HCIO(?).

According to the original description, *sori* in the ovaries, few ovaries in the panicle attacked to form dirty green, minute, round bodies up to 1 mm in diameter, half enclosed by the glumes and rupturing irregularly to expose black, dusty spore mass. *Spores* globose, subglobose, ovoid, 12.9-17.2 µm in diameter; wall medium thick, ornamented with short, blunt, triangular processes. *Sterile cells* numerous, globose to subglobose, occasionally irregular, about the size of the spores, hyaline, thick-walled, smooth.

On *Leptochloa panicea* (Retz.) Ohwi (*L. filiformis* Nees), *L. uniflora* A. Rich; S. Asia (India).

2. *Tilletia salzmannii* Maire, in Maire & Werner, 1937:47.

Type on *Koeleria salzmannii* (= *Leptochloa salzmannii*), Morocco, Moyen-Atlas, Plateau d'Ito, alt. 1450 m, VII.1921, R. Maire, MPU; isotype HUV 13106!

Sori in the ovaries, ovoid or lemon-shaped, 0.5-0.8 x 1-1.5 mm, hidden by the floral envelopes, dark brown, covered by a thin membrane of the pericarp which ruptures irregularly disclosing the dark brown, semiagglutinated to powdery mass of spores and sterile cells. *Spores* globose to broadly ellipsoidal, 18-24(-28) x 18.5-26.5(-28) µm, pale to medium yellowish-brown; wall 2.5-3.5 µm thick, usually completely, rarely incompletely reticulate, 6-10 meshes per spore diameter, muri 1-2(-2.5) µm high. *Sterile cells* globose, ovoid to irregular, 10-17 µm long, hyaline; wall even, c. 1 µm thick, smooth, content homogeneous.

On *Lophochloa salzmannii* (Boiss. & Reuter) H. Scholz (*Koeleria salzmannii* Boiss. & Reuter); N. Africa (Morocco), S. Asia (Iran).

In the original description of *T. salzmannii*, the spores were cited as 14-18 µm. These results could not be confirmed by study of the type specimen under standard conditions (spores in lactophenol, gently heated to boiling point).

3. *Tranzscheliella amplexa* (Syd.) Vánky, 2004a:109.

Ustilago amplexa Sydow, 1924:278. — Type on *Diplachne fusca*, Egypt, Belbes in the delta of Nile River, V.1880, G. Schweinfurth; isotypes Thümen, Mycoth. univ. no. 1818, HUV 3854!

Sori surrounding the upper internodes of sterile shoots, 0.2-0.3 x 10-15 cm, partly hidden by leaf sheaths, initially covered by a thin, greyish peridium which flakes away disclosing the olivaceous-brown, powdery mass of spores. *Spores* globose, subglobose, rarely ovoid or broadly ellipsoidal, flattened, in side view 4-5 µm wide, in plane view 5.5-6.5(-7) x 6-7(-7.5) µm, medium yellowish-brown; wall even, c. 0.5 µm thick, slightly thinner on the flattened sides, in LM smooth, in SEM finely, moderately densely verruculose.

On *Diplachne fusca* P. Beauv. ex Roem. & Schult.; N. Africa (Egypt).

4. *Tranzscheliella serena* (Syd.) Vánky, 2004a:109.

Ustilago serena Sydow, 1937:24. — Type on *Diplachne fusca*, Australia, New South Wales, between Warren and Collie, I.1936, L. Fraser 195; isotypes IMI 44656, HUV 17815!

Sori surrounding the uppermost culms, partly hidden by leaf-sheaths, initially covered by a delicate, grey or lead-coloured peridium which flakes away disclosing the olivaceous-brown, powdery mass of spores. *Spores* globose to broadly ellipsoidal, 6.5-8 x 7-10 μm, pale yellowish-brown; wall even, c. 0.5 μm thick, smooth in LM, finely, sparsely verruculose in SEM.

On *Diplachne fusca* P. Beauv. ex Roem. & Schult.; Australia. Known only from the type collection.

T. serena differs from *T. amplexa* especially in the larger, paler spores.

5. *Ustilago heterogena* Hennings, 1904:155.

Type on *Leptochloa virgata*, Brazil, Rio Juruá, Bom Fim, X.1900, E. Ule 2675. Topotype: 1901, E. Ule, in Mycoth. brasil. no. 3, HUV 3712!

Sori in various parts of the host plants, forming conspicuous galls or fusiform swellings on stems, leaves, inflorescence, often about 15 x 40 mm or larger, up to 2 x 12 cm, or restricted to the spikelets and as small as 0.5-1 mm in diameter, first covered by a thick, brown peridium of host tissues which ruptures irregularly at maturity disclosing the dark chocolate-brown, semiagglutinated to powdery mass of spores. *Spores* varying in shape and size, globose, subglobose, ellipsoidal, oblong, irregular, occasionally bent or with an acute tip, (7-)8-13 x 9-13.5(-15) μm, medium dark reddish-brown; wall even, c. 1 μm thick, densely low echinulate, spore profile finely serrulate.

On *Leptochloa filiformis* (Lam.) P. Beauv. (*L. mucronata* Kunth), *L. scabra* Nees, *L. virgata* (L.) P. Beauv., *L. viscida* (Scribner) Beal; N. America (Mexico, USA), S. America (Brazil).

6. *Ustilago ornata* Tracy & Earle, 1895:175.

Type on *Leptochloa mucronata* (= *L. filiformis*), USA, Mississippi, Starkville, 1.X.1894, S.M. Tracy, BPI 164358, 164361; isotypes in Ellis & Ev., N. Amer. fgi. no. 3340, HUV 9325!, HUV 4257! Topotype: 27.VIII.1895, S.M. Tracy; isotopotypes in Seymour & Earle, Econ. fgi. no. 542, HUV 7306!, and Reliquiae Seymourianae, HUV 9931!

Sori in ovaries, infecting only some in the inflorescence, ovoid, often with a short, acute tip, 0.7-1 x 1-1.8 mm, initially covered by a thin layer of the pericarp which ruptures irregularly disclosing the dark brown, semi-powdery mass of spores, or the sori fall off the plant entirely. *Spores* varying in size, globose, ovoid, ellipsoidal, rarely oblong or irregular, 9-14.5 x 10-16 μm, yellowish-brown; wall even, c. 0.5 μm thick, prominently, moderately densely echinulate, spines up to 1.5 μm high, in SEM between the spines finely verrucose.

On *Leptochloa filiformis* (Lam.) P. Beauv. (*L. mucronata* Kunth), *L. uninervia* (Presl) Hitchc. & Chase (*L. imbricata* Turb.); N. America (USA).

7. *Ustilago thaxteri* Zundel, 1939:579.

Type on *Leptochloa uninervia* (det. A. Chase), Argentina, Buenos Aires, 29.IX.1905, R. Thaxter, BPI 168169!; isotypes FH 7899, 7907, 7911, HUV 18704!

Sori destroying the whole inflorescence and extending down to and surrounding the upper portions of the stem, long cylindrical, 1-3 mm in width, 10 cm or more in length, at first partly hidden by leaf sheaths and covered by a greyish-silvery delicate membrane which flakes away revealing a semiagglutinated to powdery, olivaceous-brown spore mass. *Spores* globose, ovoid to ellipsoidal or slightly irregular, (7-)8-10.5 x 8-11(-12) µm, light olivaceous-brown; wall even, c. 0.5 µm thick, apparently smooth to very finely punctate, in SEM finely, moderately densely verrucose.

On *Leptochloa uninervia* (Presl) Hitchc. & Chase; S. America (Argentina).

A *Tilletia*, collected on *Leptochloa uniflora* A. Rich in Nyasaland (= Malawi), N.A., Kalembo, 8.VI.1955, C. Jackson 1684, represents a new species. Unfortunately, the IMI 60435 specimen contains only some spores on the envelope. Because of the paucity of the specimen, no formal description was made, only an informative one, to call attention to this bunt.

"*Tilletia leptochloae-uniflorae*" nom. prov.

Sori in the ovaries(?). *Spores* globose, subglobose, rarely ellipsoidal, 19-25 x 19-25 µm, medium to dark olivaceous-brown, provided with 2-3 µm high, cylindrical warts with flattened top, in surface view showing as darker, irregular groups, 5-8 per spore diameter, each group composed of 3-10 punctiform elements, in optical median view 18-24 warts on the equatorial circumference. *Sterile cells* globose, ellipsoidal to irregular, variable in size, 12-26 x 13.5-30 µm, pale olivaceous-brown; wall 1.5-2.5 µm thick, smooth, content granular.

Key to the smut fungi of *Leptochloa*

1. Sori in ovaries 2
– Sori elsewhere 5
2. Spores reticulate .... ***Tilletia salzmannii***
– Spores with other ornament 3
3. Spores prominently echinulate. Sterile cells absent .... ***Ustilago ornata***
– Spores with blunt or flattened warts. Sterile cells present 4
4. Spores 19-25 µm long, with cylindrical warts .... "***Tilletia leptochloae-uniflorae***"
– Spores 13-17.5 µm long, with subconical warts .... ***Tilletia leptochloae***
5(1). Sori surrounding upper internodes 6
– Sori otherwise 7
6. Spores 6-7(-7.5) µm long .... ***Tranzscheliella amplexa***
– Spores 7-10 µm long .... ***Tranzscheliella serena***
7. Sori forming conspicuous galls in various parts of the host. Spores densely low echinulate .... ***Ustilago heterogena***
– Sori in the whole inflorescence, long cylindrical. Spores apparently smooth to very finely punctate .... ***Ustilago thaxteri***

The smut fungi of *Chloris* (*Poaceae*)

Chloris Sw., in the subfam. *Chloridoideae*, tribe *Cynodonteae*, subtribe *Chloridinae*, has about 55 species in tropical and warm temperate regions of both hemispheres (Clayton & Renvoize, 1986:236). On *Chloris* at least 13 smut fungi have been described. Of these, *Tolyposporium chloridis* is a hyphomycete, *Sphacelotheca chloridis* is *Sporisorium andropogonis* on *Bothriochloa pertusa*, *Ustilago liebenbergii* is a synonym of *U. elegans*, and *Sorosporium chloridicola* is identical with *Ustilago induta*. The recognised nine species, some of them doubtful, are:

1. "*Entyloma*" *chloridis* Thirumalachar & Pavgi, 1968(1969):251.

Type on *Chloris barbata*, India, Poona, at Pimpri, 8.X.1955, M.J. Thirumalachar 1253, HCIO(?)

Sori in the leaves and leaf sheaths forming blackish, slightly raised, often coalescent, 0.5-5 mm long streaks along the veins. *Spores* agglutinated, single spores globose, subglobose, ovoid or irregularly polyhedral, 10-14.3 µm in diameter, dark olive-brown; wall thick, smooth.

On *Chloris barbata* Sw.; S. Asia (India).

This is one of the dark-spored "*Entyloma*" species on *Poaceae*, which belongs either to the genus *Jamesdicksonia*, *Phragmotaenium* or *Eballistra*, depending on spore germination and/or molecular characters. No specimen was available for study. Description taken from the original.

2. *Tilletia chloridicola* Ciferri, 1928:10.

Type on *Chloris paraguaiensis* (= *C. inflata*), Dominican Republic, Distrito Nacional, Haina, IX.1925, R. Ciferri (type where?).

Sori in the flowers and ovaries, inflated and twisted. Spore mass chestnut-brown, odourless. *Spores* globose, rarely ovoid, 12.5-15(-18) µm, brown; wall thick, densely covered with conical, pointed to subacuminate spines, 0.8-1 µm high.

On *Chloris inflata* Link (*C. paraguaiensis* Steud.); West Indies (Dominican Republic).

No material was available for study. Description taken from the original. Based on the short description, most probably it is not a *Tilletia*. It could be a *Ustilago* or a *Macalpinomyces* species.

3. *Ustilago chloridicola* Hennings, 1898:267.

Lectotype on *Chloris* sp., USA, California, Mendocino Co., Potter Valley, IV.1894, A. Purpus, (designated here) BPI 159608!; isotypes BPI 159609 & 196337.

Sori destroying the distal part of the culms including the inflorescence, 2-5 x 10-20(-30) mm, dark brown, powdery, causing shredding of the infected tissues. *Spores* variable in shape and size, globose, ovoid, long ellipsoidal, sometimes lacrymiform, (5.5-)6-9 x 6.5-11(-12) µm, olivaceous-brown; wall even, c. 1 µm thick, densely verrucose, several warts fuse, giving the spore surface an irregular pattern, spore profile wavy.

On *Chloris verticillata* Nutt., *Chloris* sp.; N. America (USA).

4. *Ustilago chloridis* Vánky, C. Vánky & R.G. Shivas, in Vánky & Shivas, 2001:171.

Type on *Chloris lobata*, Australia, Queensland, 51 km S of Lakeland, 5.III.2000, C. & K. Vánky, BRIP 27016; isotypes HUV 19177 and in Vánky, Ust. exs. no. 1084.

Sori in some ovaries of an inflorescence, lemon-shaped, 2-3 mm long. *Spores* subglobose to ellipsoidal, 9.5-13 x (10-)11-13.5(-15) µm, slightly flattened (8-11 µm wide); wall uneven, 0.5-1 µm thick, sparsely low verruculose, spore profile smooth to very finely serrulate.

On *Chloris divaricata* R. Br., *C. lobata* Lazarides, *C. truncata* R. Br.; Australia.

5. *Ustilago deserticola* Spegazzini, 1899:209.

Type on *Chloris* sp., Argentina, Salta Prov., near Amblaio, I.1897, C. Spegazzini (type where?).

Sori on the stems (internodes) and rhizomes, blackish-brown, narrow, subepidermal, causing no deformation. *Spores* globose, subpolyhedrally irregular, often compressed, 5-7 μm in diameter, blackish-brown; wall thick, smooth.

On *Chloris* sp.; S. America (Argentina). Known only from the type collection.

No material was available for study. Description taken from the original. I suspect that this is not a smut fungus.

6. *Ustilago elegans* Griffiths, 1902:292.

Type on *Chloris elegans* (= *C. virgata*), USA, Arizona, Cochise, X.1900, D. Griffiths, BPI 160338!; isotypes in Griff., W. Amer. fgi. no. 309, HUV 19955!

Ustilago liebenbergii Zundel, 1943:165. — Type on *Chloris virgata*, South Africa, Transvaal, Wolmaransstad Distr., Vlakfontein, VII.1932, L.C.C. Liebenberg, PREM 26412 & 24412; isotypes BPI 162512, HUV 15374! (syn. nov.).

Sori destroying the inflorescence, and also present within the upper 1-3 leaf sheaths (probably in induced, young inflorescence), more or less hidden by leaves and leaf sheaths, dark brown, powdery. *Spores* globose, subglobose to broadly ellipsoidal, 9.5-13 x 10-14.5 μm, laterally compressed, 7-10.5 μm wide, yellowish- to reddish-brown; wall even, 0.5-1 μm thick, moderately densely verrucose-echinulate, which just affects the spore profile. *Spore germination* (Durán, 1987:231) results in phragmobasidia with 4-5 or more cells producing hyphae.

On *Chloris submutica* H. B. K., *C. virgata* Sw. (*C. elegans* H. B. K.); Africa (South Africa), E. Asia (China), N. America (Mexico, USA).

According to Piepenbring (2003:165) probably also on *Chloris beyrichiana* Kunth (*C. radiata* Sw.) in Bolivia but the sori are forming pustules on the leaves.

No difference in the sorus and spore morphology of *Ustilago liebenbergii* and *U. elegans* could be found, hence they are considered to be synonyms.

7. *Ustilago induta* Sydow, 1939:199.

Type on *Chloris breviseta*, Sierra Leone, Njala, 12.XI.1930, F.C. Deighton 311, IMI 43068!, BPI 162017!

Sorosporium chloridicola Beeli, 1922:7 (not *Ustilago chloridicola* Henn., 1898). — Type on *Chloris polydactyla* (L.) Sw. (= misnamed *Chloris virgata*, det. J. Bosser, BR), Congo, Kinshasa, 1.VI.1916, H. Vanderyst, BR 1328!; isotype BPI 195122! (syn. nov.).

Sori in some ovaries of an inflorescence, ovoid, c. 1.5 x 2 mm, hardly evident and only slightly protruding, first covered by a leather-brown peridium enclosing a dark olivaceous-brown, powdery mass of spores. *Spores* globose, subglobose to ellipsoidal, 7.5-11 x 8-12 μm, yellowish-brown; wall even, c. 1 μm thick, sparsely verrucose, spore profile smooth to finely wavy.

On *Chloris breviseta* Benth., *C. virgata* Sw.; C. & W. Africa (Congo, Sierra Leone).

Spore balls in *Sorosporium chloridicola*, reported by Beeli (1922:7), are the result of insect work!

8. *Ustilago ulei* Hennings, 1895b:88.

Type on *Chloris* sp., Brazil, Goyaz Prov., Bom Frio, VII.1892, E. Ule 1955, HBG!; isotypes BPI 168674, BPI 168677.

Sori forming striae on the leaves, 0.5-1 x 1-30 mm, grey, covered by the epidermis which ruptures longitudinally and the dark brown, powdery mass of spores is scattered leaving behind the perforated or shredded leaves. *Spores* globose, subglobose, ellipsoidal or slightly irregular, 8-10.5 x 8-11 µm, laterally compressed, 5.5-8 µm wide, yellowish-brown; wall even, 0.5-1 µm thick, finely, moderately densely punctate-verruculose, spore profile smooth. *Spore germination* (Durán, 1987:256) results in hyphae or in 2-3-celled basidia producing basidiospores.

On *Chloris submutica* H. B. K., *C. virgata* Sw., *Chloris* sp.; N. & S. America (Mexico, Argentina, Brazil).

9. *Ustilago valentula* Sydow, 1937:24.

Type on *Chloris acicularis* (= *Enteropogon acicularis*), Australia, New South Wales, between Warren and Collie, I.1936, L.R. Fraser 194, MEL; isotypes BPI 169382, BRIP 8041, HUV 17962!, IMI 44467.

Ustilago enteropogonis Vánky, 2002a:378. — Type on *Enteropogon ramosus*, Australia, New South Wales, Bedgerebong, 'Tresta', 26.III.1982, J. Bollinger & I. McGowen, DAR 41433; isotype HUV 19540! (syn. by Vánky, 2005a:264).

Sori destroying the inflorescence, 4-8 x 15-20 mm, initially covered by a thin, greyish peridium which flakes away disclosing the dark brown, powdery mass of spores. *Spores* subglobose, ellipsoidal to slightly irregular, 9-13 x 10-14.5 µm, yellowish-brown; wall even, c. 1 µm thick, densely, minutely verruculose-echinulate, spore profile finely serrulate.

On *Chloris bournei* Rang. & Tadul., *C. pycnothrix* Trin., *Enteropogon acicularis* (Lindl.) Lazarides (*Chloris acicularis* Lindl.), *E. ramosus* B.K. Simon; NE Africa (Ethiopia), Asia, Australia.

Excluded species or not on *Chloris*

Tolyposporium chloridis is a hyphomycete

Tolyposporium chloridis Henn., in Engler, 1895c:49. — *Tolyposporidium chloridis* (Henn.) Thirumalachar & Neergaard, 1977(1978):180. — Type on *Chloris abyssinica* Hochst., Africa, "Steppe am Papyrussumpf", coll. Volkens 456.

According to Zundel, 1938:319, this is a hyphomycete.

Sphacelotheca chloridis is *Sporisorium andropogonis*

Sphacelotheca chloridis Mundkur, 1944:50. — Type on "*Chloris barbata* Sw." = misnamed *Bothriochloa pertusa* (L.) A. Camus (teste K. Vánky), India, Mysore, Bangalore, Karnataka, 20.VIII.1942, M.J. Thirumalachar, HCIO 10000; isotypes BPI 195098, HUV 17273. It is *Sporisorium andropogonis*. (syn. by Vánky, 2004b:226).

On the label of the type specimen, the original name of *Sphacelotheca bothriochloae* on *Bothriochloa pertusa* was crossed out and changed into *Sphacelotheca chloridis* on *Chloris barbata* Sw. However, study of some remnants of an infected inflorescence in the type collection revealed that the host is *Bothriochloa pertusa*, with a deep, circular pit on the lower glume of the sessile spikelet.

Key to the smut fungi of *Chloris*

(U. = Ustilago)

1. Sori on the stems or leaves forming striae 2

– Sori in the ovaries, flowers or inflorescence 4

2. Spores embedded in the host tissue, agglutinated .... **"*Entyloma*" *chloridis***

– Spores not so 3

3. Spores 5-7 µm long, smooth .... ***U. deserticola***

– Spores 8-11 µm long, punctate-verruculose .... ***U. ulei***

4. Sori in the ovaries 5

– Sori in the inflorescence 7

5. Spores 12.5-15(-18) µm long, with conical spines .... **"*Tilletia*" *chloridicola***

– Spores smaller, sparsely verruculose 6

6. Spores 8-12 µm long .... ***U. induta***

– Spores (10-)11-13.5(-15) µm long .... ***U. chloridis***

7. Spores 6.5-11(-12) µm long, irregularly verrucose .... ***U. chloridicola***

– Spores 10-14.5 µm long, regularly ornamented 8

8. Spores echinulate, spore profile serrulate .... ***U. valentula***

– Spores finely verruculose-echinulate, spore profile smooth or nearly so ***U. elegans***

The smut fungi of *Jardinea, Phacelurus* and *Rhytachne* (*Poaceae*)

Jardinea Steud. (1 sp.), *Phacelurus* Grieseb. (9 spp.) and *Rhytachne* Desv. (12 spp.) belong to the subfam. *Andropogoneae*, tribe *Rottboelliinae*. Actually, *Jardinea* is considered a synonym of *Phacelurus* (Clayton and Renvoize, 1986:363). On members of these genera five smut fungi are known. A sixth one is described below.

1. *Franzpetrakia microstegii* Thirum. & Pavgi, in Pavgi & Thirumalachar, 1957:2.

Type on *Microstegium* sp., India, Uttar Pradesh, Mussoorie Hills, IX.1954, M.S. Pavgi (type where?).

Sori destroying the whole inflorescence. The spores are mixed with long, slender, fungal filaments.

For a detailed description and illustration see Guo, Vánky & Mordue, 1990:58-62, or Vánky, 2002b:66-67.

On *Microstegium* sp. and *Phacelurus latifolius* (Steud.) Ohwi var. *monostachyus* Keng; S & SE Asia (India, China).

2. *Sporisorium rhytachnes* (Syd.) Vánky, 2000:171.

Sphacelotheca rhytachnes Sydow, 1939:202. — Type on *Rhytachne triaristata*, Sierra Leone, Mama Beach near Kent 3.XII.1936, F.C. Deighton 1109, IMI 43050!; isotypes BPI 113630, BPI 95072, HUV 17496.

The sori destroying the whole racemes, columella present. The spores are finely to prominently verruculose-echinulate.

For a detailed description see Vánky, 2000:171.

On *Rhytachne triaristata* Stapf; W Africa (Sierra Leone). Known only from the type locality.

3. ***Sporisorium rhytachnes-rottboellioidis*** Vánky, 2003:54.

Type on *Rhytachne rottboellioides*, Uganda, Masaka Distr., 13 km E Masaka, alt. 1140 m, 17.II.2002, C., T. & K. Vánky, HUV 19994; isotype in MHU, BPI, IMI, K.

Sori in all ovaries of an inflorescence, columella present. Spores finely, densely punctate.

For a detailed description and illustration see Vánky, 2003:54-55 + fig. 41.

On *Rhytachne rottboellioides* Stapf; Africa (Uganda). Known only from the type locality.

4. ***Tolyposporella rhytachnes*** Viennot-Bourgin, 1958:167.

Type on *Rhytachne minor*, Guinea (French), near Kindia, Foulaya, I.1957, G. Viennot-Bourgin, PC; isotype in HUV 15804!

Sori on the adaxial side of the rolled, tube-like leaves. Spores 9-25 µm long, wall multilayered, 2-8 µm thick, smooth. For a detailed description see Vánky, 2000:172.

On *Rhytachne minor* Pilger; W Africa. Known only from the type locality.

Amongst specimens obtained in exchange from the late Prof. Ch. Zambettakis, there is an unnamed smut fungus collected in Gabon, on *Phacelurus gabonensis*, which represents a new species:

5. ***Ustilago gabonensis*** Vánky, **sp. nov.**

Typus in matrice Phacelurus gabonensis *(Steud.) Clayton, Gabon, Moyen Ogooué, oppid. Ndjolé, c. 100 km SW Libreville, 0°07' S, 10°45' E, 10.I.1972, leg. M.G. Gilles, HUV 20999!; isotypus in BPI.*

Sori *in spiculis nonnullis sessilibus inflorescentiae eiusdem, elongate cylindrici, torti, 1-2 x 10-30 mm, plerumque 3 in eadem spicula, peridio pallide brunneo cooperti, quo longitudinaliter in locis nonnullis rupto massam sporarum mediocriter atrobrunneam, pulverescentem ostendentes. Columella nulla.* Sporae *singulae, globosae, subglobosae, ovoideae, ellipsoidales, raro elongatae vel parum irregulares, 8-11 x 8-13(-14) µm, pallide flavidobrunneae; pariete aequali, 0,5-0,8 µm crasso, verrucis vel spinis paulo sparse distributis, latis, humilibus, imago obliqua sporarum undulata vel leniter, sparse serrulata. Cellulae setriles absentes.*

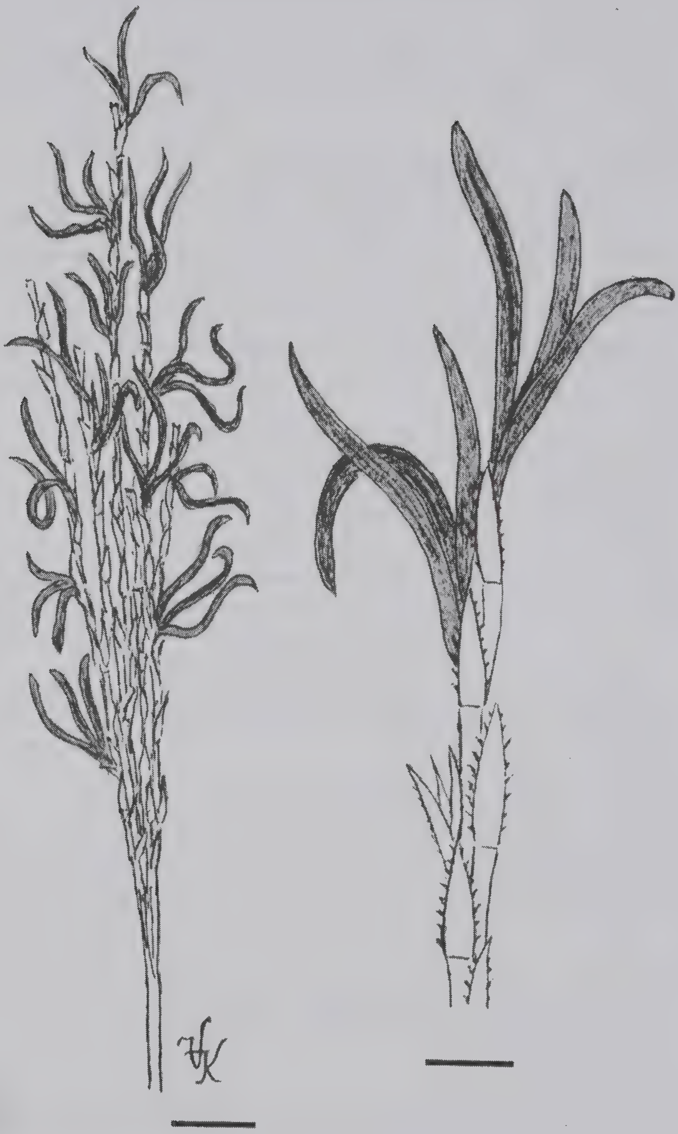
Sori (Fig. 19) in some sessile spikelets of an inflorescence, long cylindrical, twisted, 1-2 x 10-30 mm, usually three in each spikelet, covered by a pale brown peridium which splits longitudinally in several places disclosing the medium dark brown, dusty mass of spores. No columella. *Spores* (Figs. 22, 23) single, globose, subglobose, ovoid, ellipsoidal, rarely elongate or slightly irregular, 8-11 x 8-13(-14) µm, pale yellowish-brown; wall even, 0.5-0.8 µm thick, provided with rather sparsely situated, wide, low warts or spines, spore profile wavy or finely, distantly serrulate. *Sterile cells* absent.

On *Poaceae: Phacelurus gabonensis* (Steud.) Clayton (*Jardinea gabonensis* Steud.; *Rhytachne gabonensis* (Steud.) Hack.); Africa (Gabon). Known only from the type collection.

At first sight, this smut appears to be a *Sporisorium*. However, because of the lack of columella/ae, spore balls and sterile cells, it is placed in the genus *Ustilago*.

6. ***Ustilago jardineae*** (Zambett.) Vánky, **comb. nov.**

Basionym: *Cintractia jardineae* Zambettakis, Bull. Soc. Mycol. France 95:408, 1979(1980). — Type on *Jardinea gabonensis* (= *Phacelurus gabonensis*), Congo, "Mission Congo Belge". No further data. Type BR 258 (lost).



19

Fig. 19. Sori of *Ustilago gabonensis* in some sessile spikelets of *Phacelurus gabonensis* (type). Habit, and enlarged some infected and healthy spikelets.

Bars = 1 cm for habit, and 3 mm for the detail drawing.

Sori in ovaries, small, completely hidden by the glumes, ovoid or elongated, brown, agglutinated. Spore mass powdery. *Spores* globose, blackish-brown, 15-18 µm in diameter; wall 1.5-2 µm thick, granular or punctate.

Phacelurus gabonensis (Steud.) Clayton (*Jardinea gabonensis* Steud.; *Rhytachne gabonensis* (Steud.) Hack.); Africa (Congo). Known only from the type description.

No specimen was seen. The type (BR 258) is lost. It is not in BR or PC. Description based on the original and on fig. 1. in Zambettakis, 1979(1980):395. The short and incomplete original description does not allow a sure generic placement. It is certainly not a *Cintractia*, which is restricted to host plants in the *Cyperaceae*. Because no peridium, columella/ae, spore balls or sterile cells were mentioned by Zambettakis, it is recombined into the genus *Ustilago*.

Key to the smut fungi of *Jardinea, Phacelurus* and *Rhytachne*

1. Sori on the leaves. Spore wall 2-8 µm thick .... ***Tolyposporella rhytachnes***
– Sori elsewhere. Spore wall thinner 2
2. Spores intermixed with long, slender, fungal filaments . ***Franzpetrakia microstegii***
– Spores not intermixed with fungal filaments. 3
3. Sori destroying the whole racemes .... ***Sporisorium rhytachnes***
– Sori not destroying the whole racemes 4
4. Spores 15-18 µm long .... ***Ustilago jardineae***
– Spores 7.5-13(-14) µm long 5
5. Sori in all ovaries. Columella present .... ***Sporisorium rhytachnes-rottboellioidis***
– Sori in some spikelets. Columella absent .... ***Ustilago gabonensis***

Two synonyms

Pericladium flavesci is considered to be *P. grewiae*

Prasad & Tyagi (1961:500) described *Pericladium flavesci*, based on minor differences in sorus and spore germination characters compared with the common and polyphagous *P. grewiae*. According to the original description, *P flavesci* has somewhat larger and sparsely distributed sori, somewhat lower optimal temperature of spore germination, longer basidia and more globose basidiospores than *P. grewiae*. In my opinion, these differences do not justify the recognition of a separate species. Consequently, I consider *P. flavesci* a synonym of *P. grewiae*.

Pericladium grewiae Passerini, 1875:185.

Ustilago grewiae (Pass.) Hennings, 1900:(75). — Type on *Grewia* cf. *mollis*, Eritrea, near Sciotel, Zedamba, VI.1870, O. Beccari.

Pericladium flavesci Prasad & Tyagi, 1961:500. — Type on *Grewia flavescens*, India, Rajasthan, Bagdara, Udaipur, 6.XI.1958, J. Abraham., K.L. Kothari & R.N.S. Tyagi, HCIO 27004; isotype HUV 15507! (syn. nov.).

For description and illustration, including spore germination, see Vánky, 2002b:122-123.

On Tiliaceae: *Grewia asiatica* L., *G. breviflora* Benth., *G. carpinifolia* Juss., *G. columnaris* Sm., *G. flavescens* Juss., *G. microcarpa* Schum., *G. mollis* Juss., *G. orbiculata* Rottl., *G. retusifolia* Kurz, *G. venusta* Fresen, *G. villosa* Willd., *Grewia* sp.; Africa, S. Asia, Australia.

Sphacelotheca rhaphidis is *Sporisorium tumefaciens*

Sphacelotheca rhaphidis was described on *Rhaphis aciculatus*, which is a synonym of *Chrysopogon aciculatus*. On *C. aciculatus* two smut fungi are known: *Sporisorium andropogonis-aciculati* (Petch) Vánky, and *S. tumefaciens*. A comparison of the types revealed that *Sphacelotheca rhaphidis* is a synonym of *Sporisorium tumefaciens*, on *Chrysopogon pallidus*.

Sporisorium tumefaciens (McAlpine) Vánky, 1983:328.

Lectotype (designated by Vánky, 2005b:182) on "*Stipa pubescens* R. Br." = misnamed *Chrysopogon* sp. (teste S.T. Blake, in Herbert & Langdon, 1941:2, confirmed), Australia, Queensland, 40 miles S of Cloncurry, 10.V.1909, G.H. Robinson, VPRI!

Sphacelotheca rhaphidis L. Ling, 1949:128. — Type on *Rhaphis aciculatus* Retz. (= *Chrysopogon aciculatus* (Retz.) Trin.), Philippine Islands, Luzon, Manila, Wack-Wack Country Club, 10.IX.1945, C.T. Rogerson 662, BPI 113317!; isotype BPI 195059! (syn. nov.).

For its description see Vánky, 2005b:182.

On ***Gramineae:*** *Chrysopogon aciculatus* (Retz.) Trin., *C. caeruleus* (Steud.) Watson, *C. fallax* S.T. Blake, *C. latifolius* S.T. Blake, *C. pallidus* (R. Br.) Trin. ex Steud., *Chrysopogon* sp.; S. Asia, Philippines, Australia.

Miscellaneous new species

A NEW *USTILAGO* ON *PENTASCHISTIS* (*POACEAE*)

Ustilago pentaschistidis Vánky, **sp. nov.**

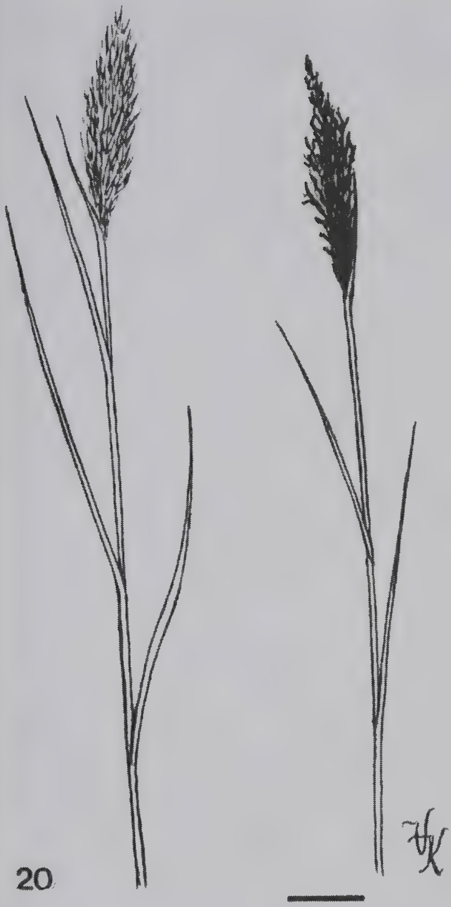
Typus in matrice Pentaschistis pallida *(Thunb.) Linder, South Africa, Western Cape Prov., Cederberg Mts., Driehoek, 32°26'21" S, 19°11'13" E, alt. 915 m, 12.X.2004, leg. R. Berndt [within the "BIOTA Southern Africa Project (SO3)"], holotypus in PREM; isotypi in HUV 20880 et in Z+ZT.*

Ustilago pentaschistidis *distincta a specie* Ustilago dregeana *L.-R. & C. Tulasne (Ann. Sci. Nat. Bot., Sér. 3, 7:83, 1847, typus in matrice* Danthonia *sp.) et a specie* Ustilago dregeanoides *K. & C. Vánky (in Vánky, Mycotaxon 65:177, 1997b, typus in matrice* Merxmuellera stricta*) imprimis teliosporis minoribus (4-5,5 µm longis), minus prominenter (leniter, humiliter tuberculatis) ornatis. Distincta etiam a specie* Ustilago sladenii *Pole-Evans (Ann. Bolus Herb. 1:115, 1915, typus in matrice* Ehrharta *sp.) imprimis teliosporis magis evidenter ornatis et plantis eius nutrientibus ad subfamiliam alteram pertinentibus.*

Sori (Fig. 20) in the whole inflorescence as naked, dark brown, powdery spore masses on the surface of short inflorescence branches, 4-5 x 20-30 mm, partly enclosed by the uppermost leaf sheath. *Spores* (Figs. 24, 25) globose, subglobose to broadly ellipsoidal, 4-5 x 4-5.5 µm, olivaceous-brown; wall c. 0.5 µm thick, slightly uneven, with two, somewhat thinner polar areas, surface finely low-tuberculate, spore profile finely to evidently wavy, more evidently at the polar areas.

On *Poaceae: Pentaschistis pallida* (Thunb.) Linder, South Africa. Known only from the type collection.

Ustilago pentaschistidis differs from *U. dregeana* and *U. dregeanoides* especially by smaller, less prominently ornamented spores. It differs from *U. sladenii*, on *Ehrharta*, especially by larger, more evidently ornamented spores and also by host plants belonging to different subfamilies.


20

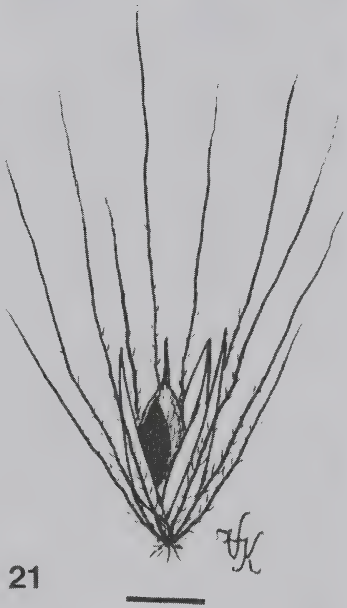

21

Fig. 20. Sori of *Ustilago pentaschistidis* in the inflorescence of *Pentaschistis pallida* (type). Habit. To the left a healthy inflorscence. Bar = 1 cm.

Fig. 21. A sorus of *Ustilago penniseti-purpurei* in the ovary of *Pennisetum purpureum* (type). Bar = 2 mm.

A new *Ustilago* on *Pennisetum* (*Poaceae*)

I revised the smut fungi of *Pennisetum* (Vánky, 2003:8-20) and recognised thirteen species. I also revised the smut fungi of other genera of the tribe *Cenchrinae*, such as *Anthephora*, *Cenchrus* and *Pseudochaetochloa* (Vánky, 2002a:391-398) and recognised eight species. Scrutinising herbarium specimens, obtained from the late Prof. Zambettakis, I found an unnamed smut fungus on *Pennisetum* "*benthamii*", which is distinct from all known smut fungi on *Cenchrinae*. It is described as:

Ustilago penniseti-purpurei Vánky, **sp. nov.**

> *Typus in matrice* Pennisetum purpureum *Schumach., Congo, c. 100 km S urbe Kinshasa (Leopoldville), oppid. Kisantu, 5°08' S, 15°09' E, 8.V.1913, H. Vanderyst 376, BR; isotypus in Herbario Ustil. Vánky, HUV 21039!*
>
> Sori *in ovariis nonnullis inflorescentiae eiusdem, longe ellipsoidales, cum apice uno acuto, cca. 1 x 2-4 mm, inter involucris floralibus prominentes, primo pericarpio sicut peridio tenui, brunneo cooperti, quo mature irregulariter rupto massam sporarum nigrescentibrunneam, semiagglutinatam usque pulveream ostendentes.* Sporae *globosae, subglobosae, ellipsoidales, saepe reniformes, 5-9 x 6,5-13,5 µm, flavidobrunneae; pariete inaequali, plerumque 0,5 µm crasso, sed in medio lateris concavi sporarum reniformium multo tenuiore, subtiliter moderate dense verruculoso-echinulato; imago obliqua sporae levis, subtiliter undulata vel ad partes tenues subtiliter serrulata.*

Sori (Fig. 21) in some ovaries of an inflorescence, long ellipsoidal with an acute tip, c. 1 x 2-4 mm, showing between the spreading floral envelopes, at first covered by the pericarp as a thin, brown peridium which ruptures irregularly at maturity disclosing the blackish-brown, semiagglutinated to powdery mass of spores. *Spores* (Figs. 26, 27) globose, subglobose, ellipsoidal, often kidney-shaped, 5-9 x 6.5-13.5 µm, yellowish-brown; wall uneven, mostly 0.5 µm thick but on the middle of the concave side of kidney-shaped spores much thinner, finely, moderately densely verruculose-echinulate, spore profile smooth, finely wavy or, on the thin-walled parts, finely serrulate.

> On *Poaceae: Pennisetum purpureum* Schumach. (*P. benthamii* Steud.); C. Africa (Congo). Known only from the type collection.

A NEW *DOASSANSIOPSIS* ON *NYMPHAEA* (*NYMPHAEACEAE*)

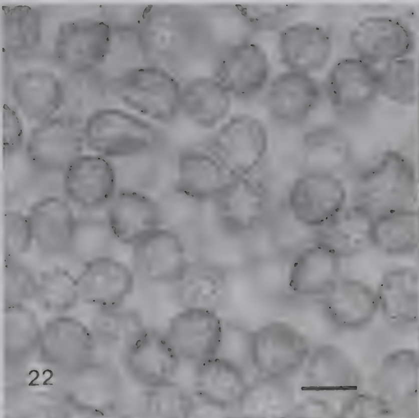
On the 50 species of *Nymphaea* of the world, three smut fungi are known: **1. *Rhamphospora nymphaeae*** D.D. Cunn. (type on *N. stellata* Willd., India), possessing solitary, lemon-shaped, hyaline spores embedded in the leaf tissue; **2. *Doassansiopsis nymphaeae*** (type on *N. stellata*, India), forming conspicuous galls on the petioles, with spore balls embedded in the host tissue; and **3. *D. ticonis*** (type on *N. blanda* G.F.W. Meyer, Costa Rica), producing flat leaf spots, containing spore balls.

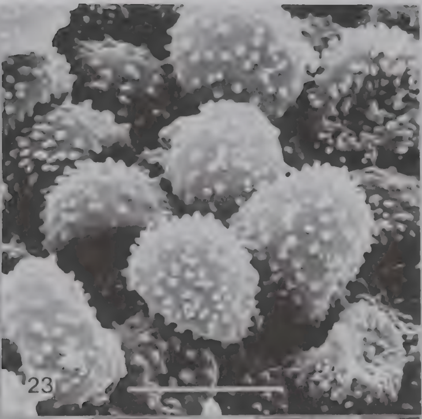
A smut fungus was collected in Ethiopia on the leaves of *Nymphaea nouchali*, producing thickened, moderately hypertrophied, wart-like or typically cup-shaped leaf spots, containing persistent spore balls. These show the typical structure of a *Doassansiopsis*, i.e., composed of a central mass of parenchymatous sterile cells surrounded by a layer of oblong, usually radially arranged, firmly adhering spores. The whole is covered by a thin cortical layer of more or less flattened, relatively small, empty fungal cells (comp. Vánky, 2002b:9, fig. 3). It is described as:

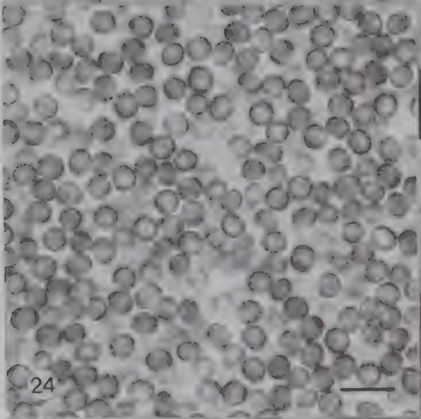
Figs. 22, 23. Spores of *Ustilago gabonensis* on *Phacelurus gabonensis*, in LM and in SEM (type).

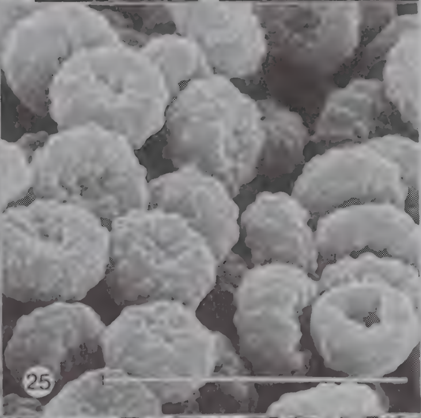
Figs. 24, 25. Spores of *Ustilago pentaschistidis* on *Pentaschistis pallida*, in LM and in SEM (type).

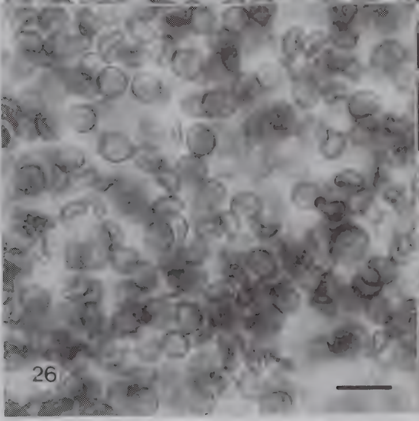
Figs. 26, 27. Spores of *Ustilago penniseti-purpurei* on *Pennisetum purpureum*, in LM and in SEM (type). Bars = 10 µm.


22


23


24


25


26

27

Doassansiopsis tomasii Vánky, sp. nov.

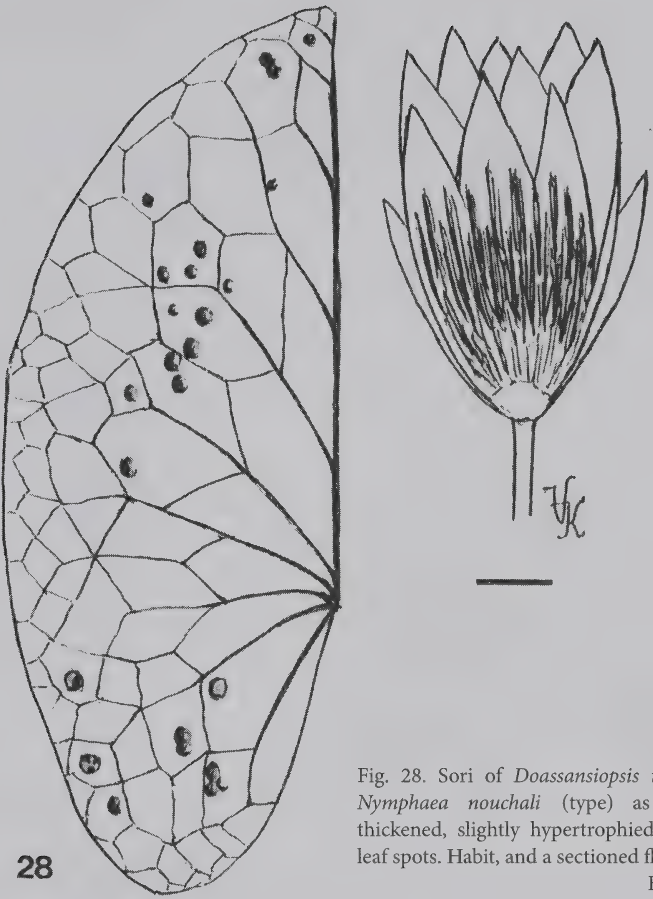
> *Typus in matrice* Nymphaea nouchali *Burm. f., Ethiopia, Gojam Reg., 17 km S urbe Bahar Dahr, haud procul Blue Nile Falls, 11°30'48.7" N, 37°29'31.6" E, alt. 1712 m.s.m., 22.X.2004, leg. T. & K. Vánky. Holotypus in Herbario Ustil. Vánky, HUV 20840!; isotypi in BPI 863739; BRIP, S et in Vánky, Ust. exs. no. 1259.*
>
> *Dassansiopsis tomasii ab utraque specie cognita generis Doassansiopsis matricis Nymphaeae distincta: D. ticonis M. Piepenbring (Mycol. Res. 99:783, 1995) et D. nymphaeae (Syd. & P. Syd.) Thirumalachar (Mycologia 39:604, 1947), basisnomine Doassansia nymphaeae H. & P. Sydow (Ann. Mycol. 10:406, 1912). A prima specie praecipue per maculas foliorum hypertrophicas productas et glomerulos sporarum maiores irregularesque, a secunda soris laminae foliorum non petiolorum et magnitudine glomerulorum sporarum distincta (cf. descriptionem accuratiorem et clavem determinationis in sequentibus).*

Sori (Fig. 28) on the leaves forming rounded, thickened, slightly hypertrophied, wart-like or cup-shaped leaf spots, evident especially on fresh material, on the abaxial side, from 1-2 mm to 5-6 mm in diameter, when dried wart-like. The spots are initially yellow, later brown, with numerous, tightly packed, pale brown (beige) spore balls embedded in the host tissue. In old sori, the necrotic host tissue is decomposed, the spore balls liberated and the leaves appear perforated. *Spore balls* varying in shape and size, globose, subglobose, ovoid, ellipsoidal, oblong or irregular, with one or several flattened sides, 130-310 x 140-400 µm, dark brown, subopaque, composed of a central mass of parenchymatous, sterile fungal cells surrounded by a layer of spores and an external, thin cortex of sterile cells. *Spores* (Fig. 31) variable in shape and size, mostly radially elongated, subpolyhedral, sometimes subcuneiform with narrowing proximal or distal part, rarely ellipsoidal, (6-)8-10.5 x (11-)13.5-21.5 µm, pale yellowish-brown, arranged in one layer, rarely two spores form the layer; wall thin, c. 0.3-0.4 µm, smooth, content homogenous. *Central parenchymatous sterile cells* (Fig. 31) extremely variable in shape and size, irregularly polyhedral with flattened contact sides, rarely ovoid or ellipsoidal, 4-21.5 x 7-32 µm, hyaline, empty; wall thin, 0.3-0.4 µm, smooth. *Cortical sterile cells* (Fig. 31) variable in form and size, irregular, radially more or less flattened, tangentially elongated, with flattened contact sides and flat, convex or hemiglobose free surface, 4-8 x 5-12 µm, subhyaline, wall 0.3-0.4 µm thick, smooth.

> On *Nymphaeaceae: Nymphaea nouchali* Burm. f. (*N. caerulea* Savigny; *N. stellata* Willd.); NE Africa. Known only from the type collection in Ethiopia, not far from the Blue Nile Falls.

Piepenbring (1995:784) describing the new species *D. ticonis,* compared it with the type of *D. nymphaeae* and wrote: "The corresponding measurements for *D. ticonis* would not provide sufficient evidence to define a new species because the size of spores and its cells tends to be rather variable in species of *Doassansiopsis*. However, the characteristics of the infected part of the host and the lack of gall growth are considered distinct enough to treat the Costa Rican collection as a new species". This statement refers also to the Ethiopian collection, a statement to which maybe geographical distribution, host plant spectrum, and some morphological differences of the spore balls and their components of the three *Doassansiopsis* species of *Nymphaea* could also be added (comp. the key below).

> *Etymology*: This fungus is named after my son, Tomas Vánky (42), physician, but an ardent and the best collector of smut fungi in the world, who found and collected so many known and unknown smuts (see HUV no. 1-21120 and the labels of Vánky, Ust. exs. no. 1-1300). He also found this new species.



28

Fig. 28. Sori of *Doassansiopsis tomasii* on *Nymphaea nouchali* (type) as rounded, thickened, slightly hypertrophied, wart-like leaf spots. Habit, and a sectioned flower. Bar = 1 cm.

Key to the smut fungi of *Nymphaeaceae*

1. On *Euryale*. Spores 16-30 µm long ..... ***Doassansiopsis euryalis***

– On *Nymphaea*. Spores shorter 2

2. Spores solitary, lemon-shaped ..... ***Rhamphospora nymphaeae***

– Spores in spore balls, not lemon-shaped 3

3. Sori as flat leaf spots, not producing hypertrophy. Spore balls globose or subglobose, 120-250 µm long. Spores 12-16 µm long. ..... ***Doassansiopsis ticonis***

– Sori producing hypertrophy. Spore balls irregular, larger. Spores longer 4

4. Sori as conspicuous swellings on the petioles. Spore balls 150-285 µm long. Spores 17-25 µm long ..... ***Doassansiopsis nymphaeae***

– Sori as thickened, wart-like or cup-shaped leaf spots. Spore balls 140-400 µm long. Spores 11-21.5 µm long ..... ***Doassansiopsis tomasii***

A NEW *USTANCIOSPORIUM* ON *RHYNCHOSPORA* (*CYPERACEAE*)

Of the 25 known species of *Ustanciosporium*, 17 are found on *Rhynchospora*. A distinct species was collected recently in the USA:

Ustanciosporium virginianum Vánky, **sp. nov.**

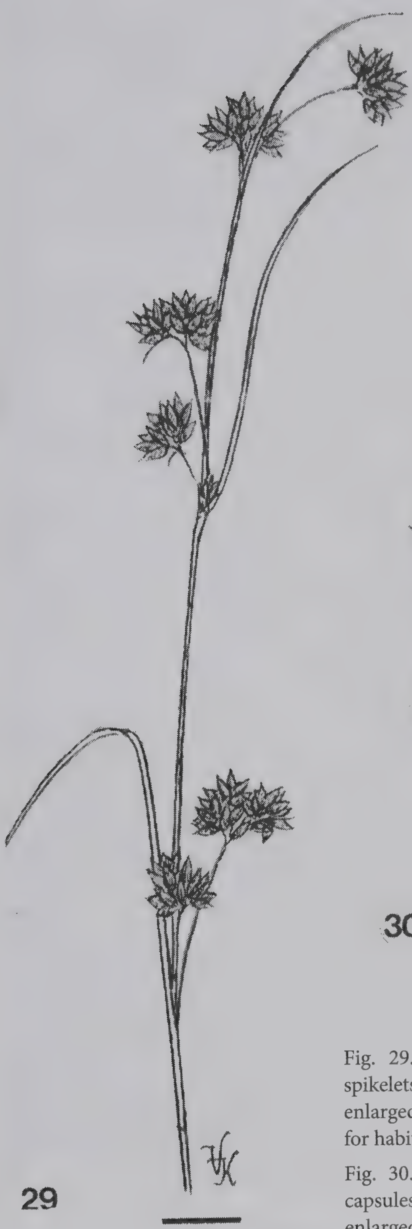
> *Typus in matrice* Rhynchospora capitellata *(Michx.) Vahl (det. T.F. Wieboldt, VPI), USA, Virginia, Giles Co., Appalachian Mts., Stony Creek Valley, via no. 635, locus dictus "Interior", 37°24'53.0" N, 80°34'51.2" W, alt. 753 m.s.m, 25.VIII.2004, leg. C. et K. Vánky. Holotypus in Herbario Ustil. Vánky, HUV 20782; isotypi in Vánky, Ust. exs. no. 1251.*
>
> Sori *in omnibus floribus inflorescentiae aliquantum congestae, massa nigra, conglutinata usque granuloso-pulverea glomerulorum sporarum atque sporarum completi, omnio glumis maxime externis occulti. Peridium et stroma sterile absentes.* Glomeruli sporarum *globosi, ovoidei, ellipsoidales, elongati vel irregulares, 25-50 x 25-80 µm, atro-rufobrunnei usque opaci, e paucis vel pluries decem sporis, pressu facile separabilibus compositi.* Sporae *parum complanatae, in visu laterali ellipticae, 7-9,5 µm latae, sine appendicibus hyalinis, in visu plano subcirculares, ovatae, ellipticae usque plerumque irregulares vel angulares, 9-13,5 x 9,5-15(-16) µm, rufobrunneae; pariete inaequali, 0,5-1(-1,5) µm crasso, leniter, dense foveolato; imago obliqua sporae levis.*

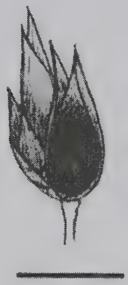
Sori (Fig. 29) in all spikelets of a somewhat congested inflorescence, completely hidden by the outermost glumes, filled with a black, granular-powdery mass of spore balls and spores. Peridium and sterile stroma lacking. *Spore balls* (Figs. 32, 34) globose, ovoid, ellipsoidal, elongated or irregular, 25-50 x 25-80 µm, dark reddish-brown to opaque, composed of a few to tens of spores which separate easily by pressure. *Spores* (Figs. 33, 34) laterally slightly compressed, in side view ellipsoidal, 7-9.5 µm, hyaline appendage lacking, in face view subcircular, ovoid, elliptic to usually irregular and angular, 9-13.5 x 9.5-15(-16) µm, reddish-brown; wall uneven, 0.5-1(-1.5) µm thick, finely, densely foveolate, spore profile smooth.

> On *Cyperaceae: Rhynchospora capitellata*; N. America (USA). Known only from the type collection.

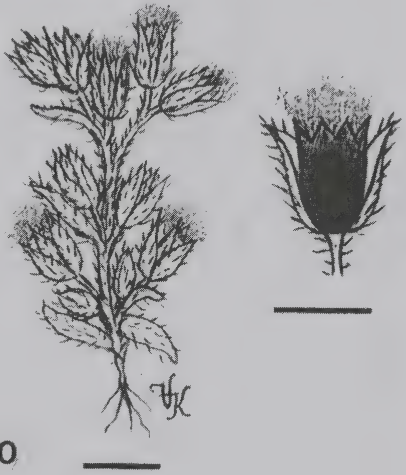
Ustanciosporium virginianum belongs to a group of smut fungi with many unsolved problems. The high frequency of "mixed" collections of diseased *Rhynchospora*, in which several „species" or „varieties" of *Ustanciosporium* are present concomitantly is surprising. The reason for this phenomenon is not clear. It caused numerous taxonomic and nomenclatorial problems and contradictions in the literature (comp. Ling, 1950, Nannfeldt, in Lindeberg, 1959:156).

The combined study of morphological and molecular characters of the genus *Cintractia* by Piepenbring, Begerow & Oberwinkler (1999), and Piepenbring (2000), resulted in the recognition of several new genera and also in a better knowledge and delimitation of numerous species. Similar investigations should be continued with as many as possible types and other specimens within the *Ustanciosporium montagnei* complex. These often differ only very little morphologically from each other. Do they represent several „small" species on the way to speciation, or one (or a few) species with high morphological variability due to hybridization, genetic variability, recombinations, segregation? A similar, unexplained case was observed by me (Vánky, 2004a:112) in a specimen of *Farysia ugandana* Zundel (= *F. butleri* (Syd. & P. Syd.) Syd. & P. Syd.) in which a part of one sorus contains much darker, somewhat agglutinated, almost smooth to finely punctate spores instead of the typical olive-brown, dusty, finely, distinctly verruculose spores of the species.





29



30

Fig. 29. Sori of *Ustanciosporium virginianum* in all spikelets of *Rhynchospora capitellata* (type). Habit, and enlarged a longitudinally sectioned spiklet. Bars = 1 cm for habit, and 2 mm for the detail drawing.

Fig. 30. Sori of *Microbotryum afromontanum* in the capsules of *Cerastium afromontanum* (type). Habit, and enlarged a longitudinally sectioned capsule. Bars = 5 mm for habit, and 3 mm for the detail drawing.

A NEW *MICROBOTRYUM* ON *CERASTIUM* (*CARYOPYLLACEAE*)

Microbotryum afromontanum Vánky, sp. nov.

Typus in matrice Cerastium afromontanum *T.C.E. Fr. & Weimark, Ethiopia, Gondar Reg., 62 km NE pag. Debart, Simien Mountains, 13°15'29.1" N, 38°12'58.1" E, alt. 4060 m.s.m., 25.X.2004, leg. T. & K. Vánky. Holotypus in Herbario Ustil. Vánky, HUV 20888; isotypi in BPI 863704, S et in Vánky, Ust. exs. no. 1265.*

Sori *ovula destruentes, capsulas massa sporarum purpurascentibrunnea, primo agglutinata serius pulverea implentes. Infectio systemica, capsulas omnes plantae eiusdem inficiens.* Sporae *globosae, subglobosae usque late ellipsoidales, 11-14,5 x 12-16 µm, pallide flavidobrunneae, pallide violaceo tinctae; pariete subtiliter, dense, nonnunquam incomplete reticulato, (5-)6-9 maculis in diametro sporae, muri earum 0,8-1,5 µm alti, acuti, subacuti vel obtusi, 21-30 in circumferentia aequatoriali.*

Sori (Fig. 30) destroying the ovules filling the capsules with a purplish-brown, at first agglutinated, later powdery spore mass. Infection systemic, all capsules of a plant being infected. *Spores* (Figs. 35, 36) globose, subglobose to broadly ellipsoidal, 11-14.5 x 12-16 µm, light yellowish-brown with a pale violet tint; wall finely, densely, sometimes incompletely reticulate, (5-)6-9 meshes per spore diameter, muri 0.8-1.5 µm high, acute, subacute or blunt, 21-30 on the equatorial circumference.

On *Caryophyllaceae* (subfam. *Alsinoideae*): *Cerastium afromontanum* T.C.E. Fr. & Weimark, NE. Africa (Ethiopia). Known only from the type collection.

Microbotryum afromontanum differs from *M. duriaeanum* (Tul. & C. Tul.) Vánky (type on *Cerastium glomeratum* Thuill., Algeria) especially in spores having more and smaller meshes and lower muri. *M. duriaeanum* (on *Cerastium brachypetalum* Pers., Vánky, Ust. exs. no. 112) has 4-7 meshes per spore diameter, muri 1-2.5 µm high, acute or subacute, 16-22 on the equatorial circumference.

A NEW *SPORISORIUM* SPECIES ON *ECHINOCHLOA* (*POACEAE*)

Ingold (1996) demonstrated that five collections of smut fungi, identified as *Ustilago trichophora* on *Echinochloa* spp., originating from HUV, show two different types of spore germination. Since then, I intended to find out the cause of this phenomenon. In my efforts to identify a specimen recently collected in Ethiopia on *E. colona*, I investigated all 63 collections of *U. trichophora* in HUV. The result is that besides *U. trichophora*, there is an apparently very similar but distinct species belonging to the genus *Sporisorium* which is described as:

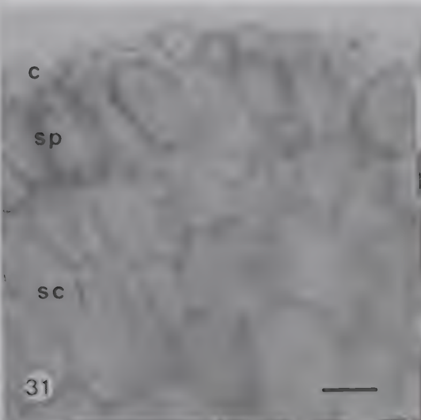
Sporisorium ingoldii Vánky, sp. nov.

Typus in matrice Echinochloa colona *(L.) Link, India, Uttar Pradesh, Varanasi, Banaras Hindu University Campus, 25°20' N, 83°00" E, alt. cca. 100 m.s.m., 4.X.1992, leg. C. & K. Vánky. Holotypus in Herbario Ustil. Vánky, HUV 15764; isotypi in Vánky, Ust. exs. no. 948 (ut* Ustilago trichophora*). Paratypi in matrice E. colona, India, Madhya Pradesh, 21*

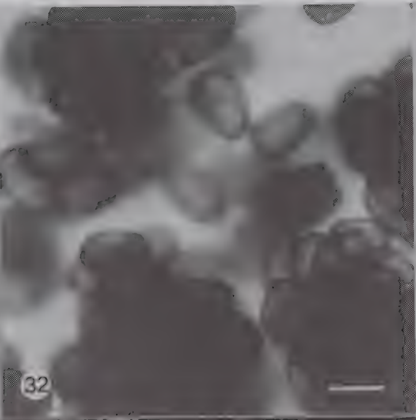
Fig. 31. Part of a spore ball with spores (sp), sterile cells (sc) and cortex (c) of *Doassansiopsis tomasii* on *Nymphaea nouchalii*, in LM (type).

Figs. 32, 33, 34. Spore balls and spores of *Ustanciosporium virginianum* on *Rhynchospora capitellata* in LM and in SEM (type).

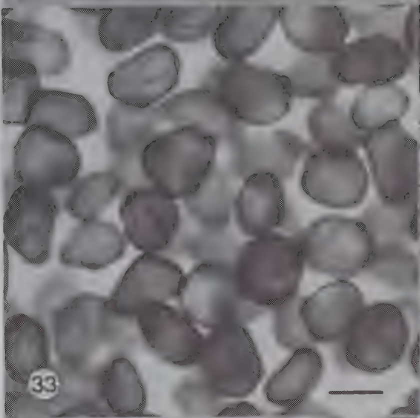
Figs. 35, 36. Spores of *Microbotryum afromontanum* on *Cerastium afromontanum*, in LM and in SEM (type). Bars = 10 µm.



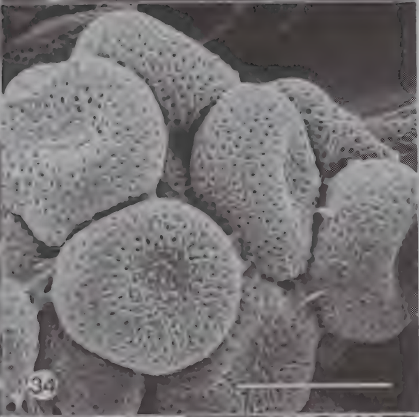
31



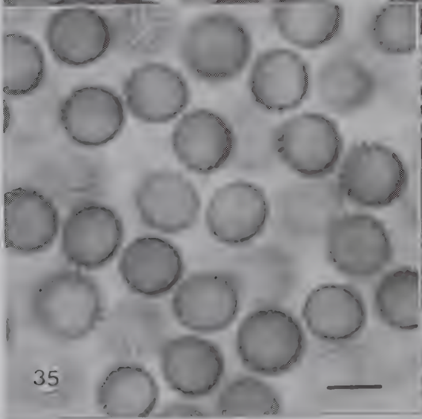
32



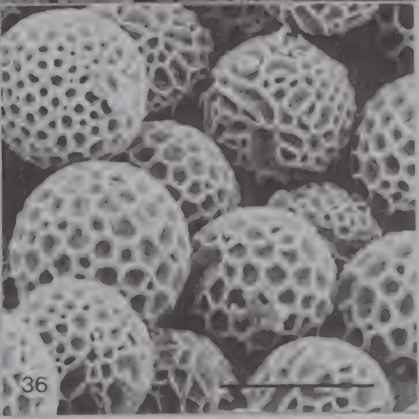
33



34



35



36

km NW urbe Jabalpur, 15.X.1992, N.D. Sharma, R.S. Singh & K. Vánky, HUV 15587. E. colona, *Ethiopia, Gondar Reg., supra Blue Nile Falls, 11°30'48" N, 37°29'30" E, alt. 1710 m.s.m., 22.X.2004, leg. T. & K. Vánky, HUV 20936; isoparatypi in Vánky, Ust. exs. no. 1270.*

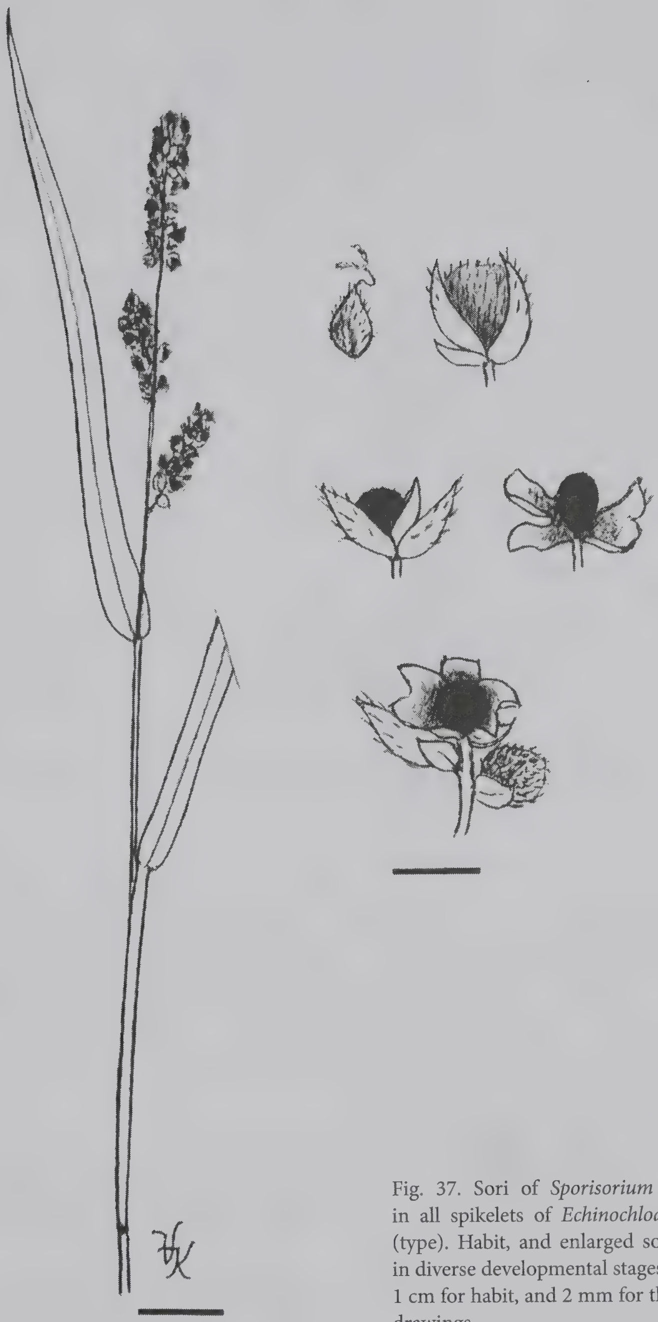
Sporisorium ingoldii *distinctum a specie manifeste simili* Caeoma trichophorum *Link (in Linné's Species Plantarum, Ed. 4, 6(2):3, 1825,* = Ustilago trichophora *(Link) Körn.) imprimis characteribus soralibus et typo germinationis sporarum. In specie* Sporisorium ingoldii *plerumque spiculi omnes eiusdem inflorescentiae affecti, peridium in maturitate cito ruptum, columella brevis, crassa praesens, et saepe etiam catervae cellularum sterilium praesentes. In specie* Ustilago trichophora *spiculae singulae vel catervae earum affectae, peridium satis persistens, columella et cellulae steriles absentes. Praeterea sori speciei* Sporisorium ingoldii *spiculis restricti, sed illi speciei* Ustilago trichophora *saepe etiam in culmis evoluti. Distinctiones in germinatione sporarum a C.T. Ingold (1996:418-420, fig. 1-7) descriptae et illustratae.*

Sori (Fig. 37) usually in all spikelets of an inflorescence, globoid or rarely bilobed, 1-2 mm in diameter, partly hidden by floral envelopes, first covered by a thick, hispid peridium which ruptures irregularly at maturity disclosing the dark brown, semiagglutinated to powdery mass of spores and sterile cells surrounding a thick, short, simple columella with longitudinal furrows. *Spores* (Figs. 40, 41) single when mature, globose, subglobose, ovoid to broadly ellipsoidal, 8-10.5 x 9-12 µm, yellowish-brown; wall even, c. 0.5 µm thick, sparsely low echinulate, spore profile wavy to finely, distantly low serrulate. *Sterile cells* few or lacking, when present in small, irregular groups, single cells globoid, ellipsoidal to irregular, with one or several flattened sides, 4.5-9 x 5.5-12 µm, hyaline; wall c. 0.5 µm thick, smooth. *Spore germination* (Ingold, 1996) results in a long, slender, 4-celled basidium measuring 1.5-2 x 40-60 µm, laterally and terminally giving rise to ovoid or cylindrical basidiospores on short sterigmata.

On *Echinochloa colona* (L.) Link; Africa (Ethiopia, S. Africa, Zimbabwe), S. Asia (India, Pakistan). Probably more common but overlooked or identified as *Ustilago trichophora.*

Sporisorium ingoldii is apparently similar to *Ustilago trichophora*. The most evident difference lies in the sorus characters and in the spore germination. In both species the sori are covered by a hispid peridium. However, in *S. ingoldii* usually all spikelets of an inflorescence are affected (systemic infection?), the peridium ruptures early at maturity, there is a short, stout columella and often also groups of sterile cells, whereas in *U. trichophora* only single spikelets or groups of spikelets are affected, the peridium is rather persistent, a columella is lacking (rarely, irregular host tissue remnants may give the appearance of a columella) and no sterile cells are present. In addition, in *S. ingoldii* the sori are restricted to the spikelets only whereas in *U. trichophora* they can be present also on the stems and leaves. The spores in both species look very similar but in *U. trichophora* they are slightly smaller (6-11 x 7-12 µm). Differences in spore germination are described and illustrated by Ingold (1996:418-420, figs. 1-7). It seems, that *S. ingoldii* is restricted to *Echinochloa colona*, whereas *U. trichophora* infects many *Echinochloa* species, including *E. colona.*

Etymology: This species is dedicated to Professor C. Terence Ingold, at the occasion of his 100th birthday (3 July 2005), a many-sided, most outstanding contemporary mycologist, scientist, teacher and a warm-hearted, inspiring human being and friend. I am happy for the privilege of the over 20 years of correspondence and co-operation with him.



37

Fig. 37. Sori of *Sporisorium ingoldii* in all spikelets of *Echinochloa colona* (type). Habit, and enlarged some sori in diverse developmental stages. Bars = 1 cm for habit, and 2 mm for the detail drawings.

A NEW *USTILAGO* ON *SETARIA* (*POACEAE*)

Ustilago trichogena Vánky, sp. nov.

> *Typus in matrice* Setaria setosa *var.* leiantha *Hack., Bolivia, Dept. Tarija, Tarija, 21°33' S, 64°45' W, 6.II.1902, leg. R.E. Fries 271, S; isotypus in HUV 5214!*
>
> Ustilago trichogena *a* Caeoma trichophorum *Link, in Linné's Species Plantarum, Ed. 4, 6(2):3, 1825 [= U. trichophora (Link) Körn., typus in matrice* Panicum colonum *L., Egypt], distincta soris acutis, sporis irregularibus longioribusque, 5,5-8(-9) x 6,5-16 μm, et superficie sporarum levi vel valde leniter, parce granulosa inter verrucas humiles vel spinas in SEM conspicuas.*

Sori (Fig. 38) in some flowers of an inflorescence, transforming the ovaries and filaments into fusiform or ovoid bodies with acute tip, 1-2 x 2-3 mm, covered by a thick, brown, hairy peridium, protruding between the floral envelopes, of which the glumes are intact, the other ones are often more or less hairy. At maturity the peridium ruptures irregularly disclosing the dark brown, semiagglutinate to powdery mass of spores. *Spores* (Figs. 42, 43) rarely subglobose, usually slightly irregular, ovoid, ellipsoidal, often considerably elongated, sometimes even slightly bent, 5.5-8(-9) x 6.5-16 μm, yellowish-brown; wall even, c. 0.5 μm thick, sparsely, finely verrucose-echinulate, spore profile smooth to finely wavy; in SEM, between the low warts or spines the surface is smooth or very finely, sparsely granular. *Sterile cells* absent.

> On *Setaria setosa* var. *leiantha*; S. America (Bolivia). Known only from the type collection.

Ustilago trichogena is close to *U. trichophora*, type on *Panicum colonum* L. (= *Echinochloa colona* (L.) Link), Egypt. In both species the sori are hispid. However, in *U. trichophora* the sori are more globose, and often appear also on the stems, the spores are more regular, rounded, measuring 6-11 x 7-12 μm, and the surface between the spines is sparsely verruculose as seen in SEM.

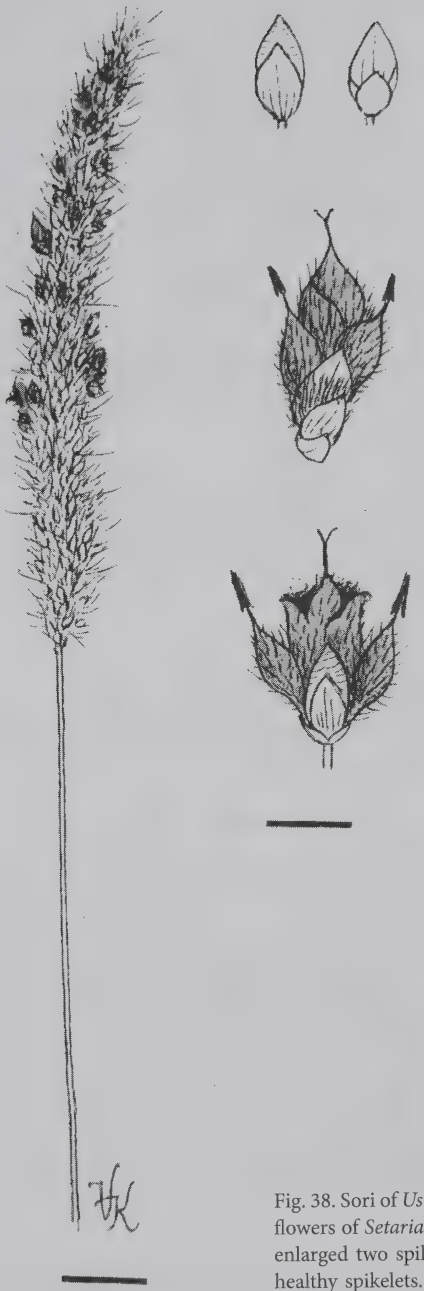
> *Etymology: trichogena* from Greece *thrix* = hair, and –genes = producing, referring to the hairy soral membrane, produced by the fungus.

A SECOND SPECIES OF *PILOCINTRACTIA* ON *FIMBRISTYLIS*

The genus *Pilocintractia* Vánky (2004d:172) was erected for *Cintractia fimbristylidicola* Pavgi & Mundk. (type on *Fimbristylis complanata* Link, India), based on molecular biological data and the peculiar soral structure. A smut fungus, collected by Dr. N.D. Sharma (Jabalpur, India), and another one, obtained in exchange from HCIO, under the name of *Cintractia indica* Pavgi & Mundk., nom. herb., on „*Fimbristylis aestivalis*" (Retz.) Vahl (= misnamed *F. miliacea*), have similar soral structure but larger spores. They both represent the same species and are described as:

Pilocintractia adrianae Vánky, sp. nov.

> *Typus in matrice* Fimbristylis miliacea *(L.) Vahl (det. K. Vánky), India, Madhya Pradesh, cca. 115 km W urbe Jabalpur, pag. Bohani, X.1977, leg. N.D. Sharma, HUV 20947!; isotypi in BPI et HCIO. Paratypus in matrice* Fimbristylis miliacea *(det. K. Vánky, conf. K.A. Lye, NLH), India, Punjab, Amritsar, 31°35' N, 74°56' E, 12.X.1907, leg. A. Hafiz Khan, HUV 15463!; isoparatypus in HCIO 1437.*
>
> Sori *corpora atrobrunnea, globoidea, ovoidea, compacta, dura, diametro 1-1,5 mm circa nuces nonnullas spiculorum aliquorum eiusdem inflorescentiae formantes. Peridium*



38

Fig. 38. Sori of *Ustilago trichogena* in some flowers of *Setaria setosa* (type). Habit and enlarged two spikelets with sori and two healthy spikelets. Bars = 1 cm for habit, and 2 mm for the detail drawings.

eorum deest, superficie non pulverei. Sori maturi in unum continentes a planta nutrienti decidentes. Sporae *complanatae, a latere ellipticae, 8-10,5 μm latae, sine appendicibus hyalinis, in aspectu plano circulares, subcirculares, ellipticae usque parum irregulares, 11-14,5 x 11.5-16(-17) μm, rubrobrunneae; pariete aequali, cca. 0,5 μm crasso, conspicue levi usque valde subtiliter, dense punctato-verruculoso. Sporae in marsupiis cupulatis formatae. Inter sporis filamenta fungalia, hyalina, sterilia, cum muris gelatinosis praesentes, sporas maturas conglutinantia. Germinatio sporarum non cognita.*

Sori (Fig. 39) forming dark brown, globoid, ovoid, compact, hard bodies around some nuts in several spikelets of an inflorescence, 1-1.5 mm in diameter. Peridium lacking, surface not powdery. Mature sori fall off the plants in one piece. *Spores* (Figs. 44, 45) flattened, in side view elliptic, 8-10.5 μm wide, no hyaline appendages, in plane view circular, subcircular, elliptic to slightly irregular, 11-14.5 x 11.5-16(-17) μm, reddish-brown; wall even, c. 0.5 μm thick, apparently smooth to very finely, densely punctate-verruculose. The spores are formed in cup-shaped pockets. Among the spores hyaline, sterile, fungal filaments with gelatinised wall are present, gluing the spores together. *Spore germination* unknown.

On *Cyperaceae: Fimbristylis miliacea*; S. Asia (India). Known only from the type collections.

Etymology: This species is named in the memory of the young, talented, Colombian biologist, Adriana Mercedes Gill Correa, married in Germany, collector of numerous interesting, rare and also new neotropical smut fungi (comp. Piepenbring, 2002b, 2003), whose lost fight against a malignant tumour that prevented her making a great mycological career.

Key to the *Pilocintractia* species

– Spores 9-12(-13) μm long, finely granular-verruculose............ ***P. fimbristylidicola***

– Spores 11.5-16(-17) μm long, apparently smooth to finely punctate.. ***P. adrianae***

New combinations

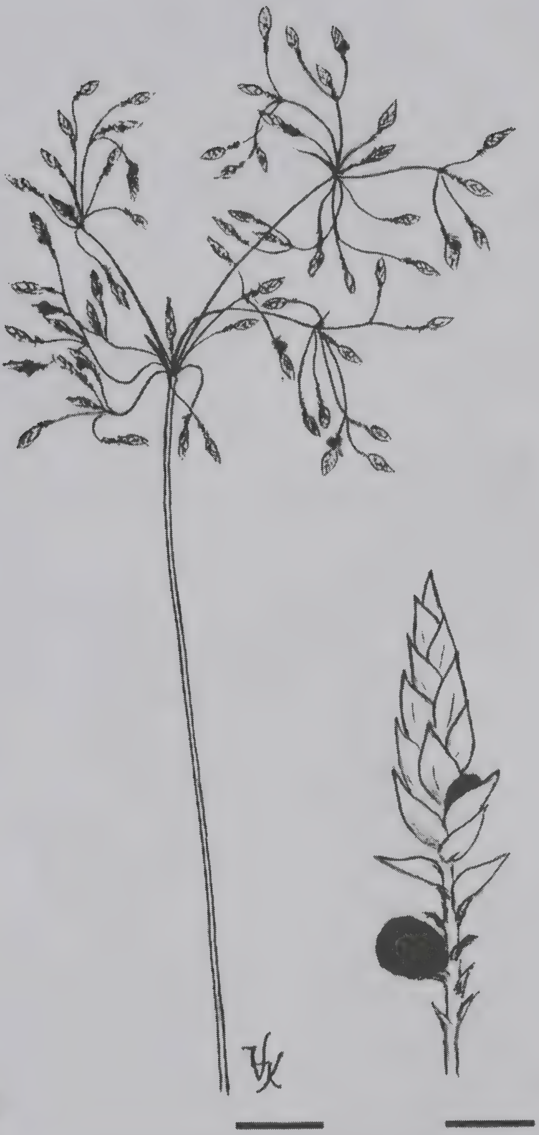
A further member of the genus *Heterodoassansia* (*Doassansiaceae*)

Heterodoassansia Vánky (1993:28) differs from the genus *Doassansia* Cornu (1883:283) in the heterogeneity of the cortical sterile cells of the spore balls (see below). It has five known species. Recently, smutted leaves of *Hygrophila auriculata* were collected in Ethiopia, with spore balls embedded in the host tissue. Study of thin, hand-cut slices of the spore balls revealed that they represent the same species which was known only from India, under the name of *Doassansia hygrophilae*. It turned out also, that this species belongs to the genus *Heterodoassansia*:

Heterodoassansia hygrophilae (Thirum.) Vánky, **comb. nov.**

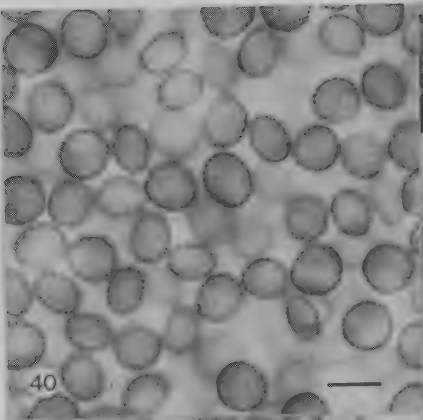
Basionym: *Doassansia hygrophilae* Thirumalachar, Lloydia 9:29, 1946. — Type on *Hygrophila* sp. (= *H.* cf. *auriculata*, teste K. Vánky), India, Mysore, Nandi Hills, 5.XI.1944, M.J. Thirumalachar, HCIO 10692; isotypes BPI 178386, 178388, 195045, IMI, HUV 15470!

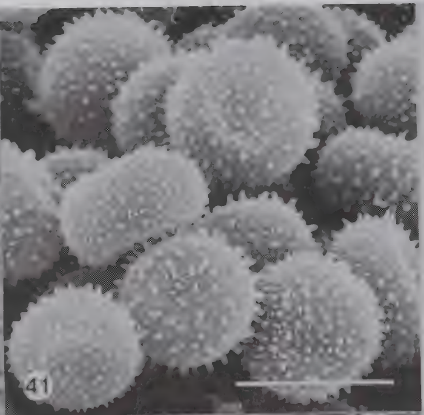
Sori on leaves forming initially yellow, later brown, circular spots, 2-10 mm in diameter or larger by confluence, with permanent spore balls embedded in the host tissue as minute, dark brown, raised, mainly hypophyllous dots. Later the leaves may be perforated. *Spore*

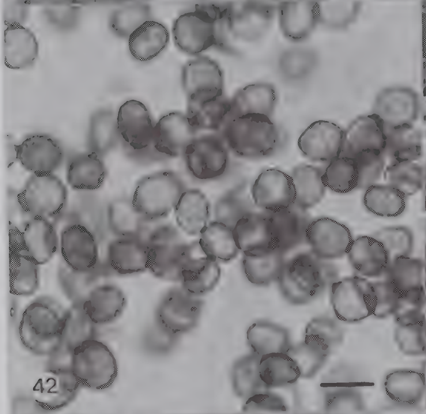


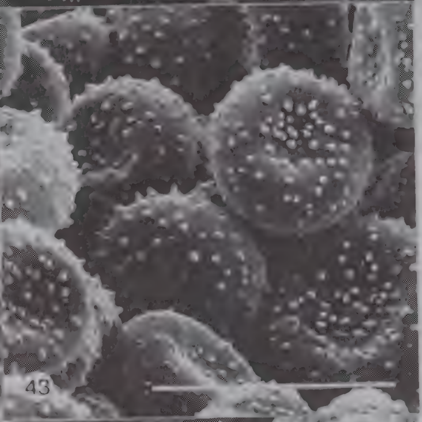
39

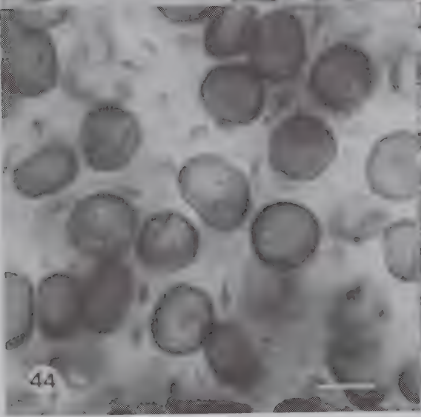
Fig. 39. Sori of *Pilocintractia adrianae* around some nuts of *Fimbristylis miliacea* (type). Habit and enlarged a spikelet with two sori. Bars = 1 cm for habit, and 1 mm for the detail drawing.

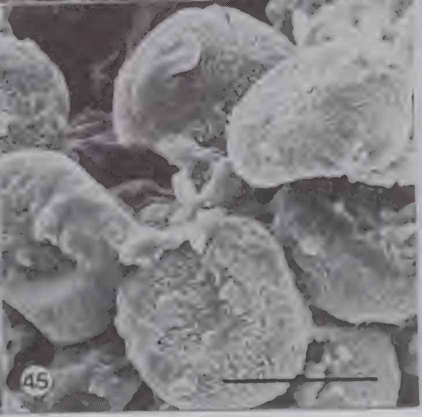

40


41


42


43


44


45

balls globose, subglobose, ovoid to slightly irregular, 100-170 x 110-200 µm, beige-brown, composed of a central mass of loosely aggregated spores surrounded by a firm cortex of two different kinds of sterile cells. *Spores* subglobose, ellipsoidal to slightly irregular, 7-12 x 9-16 µm, subhyaline, easily separating by pressure; wall even, thin (c. 0.5 µm), smooth. *Cortex* composed of an inner layer of radially oblong, tightly adhering, relatively large, irregularly subpolyangular, empty cells measuring 6-18 x 7-25 µm, pale yellowish-brown; wall even, c. 0.5 µm thick, ornamented on its inner surface (towards the empty lumen) by moderately densely situated, thin, 0.5-1.5 µm high spines. These cells are arranged in one layer, which in some places is formed by two smaller cells. The outer layer of the cortex is the last-formed component of the spore balls. It differentiates from tangentially elongated, thin-walled, septate, plasmatic fungal filaments. When mature, this thin covering layer is formed of small, irregularly polyangular, often radially flattened, 5-12 µm long, pale yellow, empty cells with c. 0.4 µm thick wall, smooth on the inner and outer surface. *Spore germination* (Thirumalachar, 1946) results in holobasidia bearing an apical whorl of 5-7, fusiform, slightly asymmetrical basidiospores measuring 1-1.2 x 14-15 µm. These conjugate two by two forming binucleate infection hyphae. On the hyphae, on well-developed sterigmata, short, slightly bent ballistospores can be produced.

On *Acanthaceae: Hygrophila auriculata* (Schumach.) Heine (*H. spinosa* T. Anders.; *Asteracantha longifolia* (L.) Nees); Africa (Ethiopia), Asia (India).

Some *Sporisorium* species

Andropterum Stapf is a unispecific genus in the subfam. *Andropogoneae*, tribe *Ischaeminae*, with the type *A. variegatum* Stapf (= *A. stolzii* (Pilger) C.E. Hubb.), in Central Africa (Clayton & Renvoize, 1986:348). Scrutinising herbarium specimens obtained from the late Prof. Zambettakis, I found two sori of an unnamed smut fungus, originating from BR. It turned out to be the isotype of *Sphacelotheca andropteri*, which belongs to the genus *Sporisorium*.

Sporisorium andropteri (Zambett.) Vánky, **comb. nov.**

Basionym: *Sphacelotheca andropteri* Zambettakis, Bull. Soc. Mycol. France 95:410, 1979(1980). — Type on *Andropterum stolzii*, Congo, S of Boudouinville, Mission St. Martin, Valley of Cumons, leg. Boudewyn 51, BR 2!

Sori in sessile and pedicelled spikelets, cylindrical, 0.8-1 x 3-5 mm, partly hidden by the floral envelopes, covered by a thick, brown peridium which ruptures irregularly disclosing the blackish-brown, first agglutinated later dusty mass of spores and groups of sterile cells surrounding a simple, stout, narrowing central columella. *Spores* single when mature, globose, subglobose, ovoid or broadly ellipsoidal, 4.5-5.5 x 4.5-6.5 µm, yellowish-brown; wall uneven, c. 0.5 µm thick with a thinner part on one side, in LM apparently smooth to very finely punctate, in SEM moderately densely low verrucose. *Sterile cells* in irregular groups or chains, rarely solitary, single cells subglobose, ellipsoidal

---

Figs. 40, 41. Spores and sterile cells of *Sporisorium ingoldii* on *Echinochloa colona*, in LM and in SEM (type).

Figs. 42, 43. Spores of *Ustilago trichogena* on *Setaria setosa*, in LM and in SEM (type).

Figs. 44, 45. Spores and gelatinised fungal filaments of *Pilocintractia adrianae* on *Fimbristylis miliacea*, in LM and in SEM (type). Bars = 10 µm.

to usually irregular, with one or several flattened sides, 8-14 µm long, subhyaline to pale yellowish-brown; wall 0.8-1.5 µm thick, smooth.

> On *Poaceae: Andropterum stolzii* (Pilger) C.E. Hubb.; Africa (Congo). Known only from the type collection.

Revising the smut fungi of *Ischaemum* (Vánky, 2004a:94-102), I recognised 11 species. A further species, *Sorosporium semisagittatum*, was also given with its original description, because no material of it was available for study (the type probably being lost). Identifying smut fungi of *Ischaemum indicum*, collected in India, Madhya Pradesh, Kundam, 11.X.1992, leg. N.D. Sharma, C. & K. Vánky (HUV 21078), and Maharashtra State, near Khandala, 25.X.1992, leg. C. & K. Vánky (HUV 21077), it turned out that these specimens represent *S. semisagittatum*, which belongs to the genus *Sporisorium*. The second collection is also distributed in Vánky, Ust. exs. no. 1290.

Sporisorium semisagittatum (Thirum. & Pavgi) Vánky, **comb. nov.**

> Basionym: *Sorosporium semisagittatum* Thirum. & Pavgi, Sydowia 20:22, 1967(1968). — Type on *Ischaemum semisagittatum*, India, Maharashtra State, Poona, Khandala, 14.XI.1954, M.J. Thirumalachar 1235 (type where?).

Sori in some spikelets of an inflorescence, ellipsoidal or short cylindrical, 0.8-1.5 x 3-5 mm, showing between the spreading floral envelopes, initially covered by a thick, pale brown peridium which ruptures irregularly from its apex disclosing the blackish-brown, first agglutinated later dusty mass of spore balls, spores and groups of sterile cells surrounding a stout, narrowing central columella, often with shortly bi- or trifurcate tip or with short lateral branches. *Spore balls* subglobose, ellipsoidal, oblong or irregular, of varying size, 30-90 x 35-120 µm, dark reddish-brown to opaque, composed of tens to hundreds of spores which separate easily by pressure. *Spores* globose, subglobose, ovoid ellipsoidal, oblong or rounded subpolyhedrally irregular, 7-9 x 8-11(-12) µm, yellowish-brown; wall slightly uneven, 0.5-0.8 µm thick, densely verrucose-echinulate, spore profile smooth, finely wavy to finely serrulate. *Sterile cells* rather few, usually in rounded, compact groups of (3-)5-10(-15?), single cells irregular, with one or several flattened sides, 5-11 µm long, subhyaline to pale yellowish-brown; wall c. 0.5 µm thick, smooth.

> On *Poaceae: Ischaemum indicum* (Houtt.) Merr. (*I. ciliare* Retz.), *I. semisagittatum* Roxb.; S. Asia (India).

Three additional synonyms

Entyloma lavrovianum is a synonym of *E. hieracii*

Entyloma lavrovianum Schwarzman (1960:297) was described from Kazakhstan on several collections of two *Hieracium* species (no type designated). Two syntypes, one on *H. ganeschinii* Zahn, another on *H. korshinskyi* Zahn, both from Alma-Ata Region, Zailijskij Alatau, Great Alma-Ata Gorge, 19.VIII.1958, C. Schwarzman, AA, HUV 12112! & HUV 12113!, were compared with the lectotype of *Entyloma hieracii* Syd. & P. Syd. ex Cif., on *Hieracium murorum* L., Germany, HUV 1174! No differences in spore morphology (shape, size, wall thickness) could be evidentiated, excepting a somewhat darker colour of the spores of *E. lavrovianum*, namely pale yellow to very pale yellowish-brown. The spores of the type of *E. hieracii* are subhyaline to pale yellow. Therefore, I am considering the two names synonyms.

Leucocintractia leucodermoides is considered to be *L. leucoderma*

Leucocintractia leucodermoides M. Piepenbr. & Begerow, was described in Piepenbring (2000:315), based on some differences in spore measurements, spore wall thickness and colour of the spores as compared with the similar *L. leucoderma* (Berk.) M. Piepenbr. A comparison of the types could reveal only minor differences, less than was given in the original description. For the length of the spores of *L. leucoderma* I obtained 14.5-21 µm (instead of 17-19(-21) µm), and for the spore wall thickness 1.5-3 µm. For *L. leucodermoides* these were 13-20(-21) µm (instead of (14-)15-18(-19 µm), and 1.5-2.5 µm, respectively. The difference of the spore ornamentation between these two species, seen in SEM, was also minimal. Consequently, I am considering these two species to represent only variation within the same species, *L. leucoderma*. (syn. nov.).

Tilletia cynodontis is a synonym of *T. montemartinii*

Tilletia montemartinii Canonaco (1936:35) was described on *Cynodon glabratus* Steud. (= *C. dactylon* (L.) Pers.) from Erythrea. It was forgotten and did not appear in recent literature. I described *T. cynodontis* Vánky (2001:294) on *Cynodon plectostachyus* (K. Schum.) Stapf from Ethiopia. No specimen of *T. montemertinii* was available for study but based on the description, I consider the two names to by synonyms. For description and illustration (as *T. cynodontis*) see Vánky, 2001:294, fig. 26, and figs. 24, 25, p. 291.

Acknowledgements

I am grateful to S. Tóth (Gödöllő, Hungary) for preparing the Latin diagnoses, to E.H.C. McKenzie (Auckland, New Zealand), and R. Berndt (Zürich, Switzerland) for reading the manuscript and serving as pre-submission reviewers. Many thanks to N.D. Sharma (Jabalpur, India) for smut fungus specimens, to K.A. Lye (NLH, Norway), H. Scholz (B, Germany) and T.F. Wieboldt (VPI, USA) for identifying host plants. Thanks are also due to the Directors and Curators of the Herbaria BPI, BR, BRIP, HCIO, IMI, PREM and S for loans and/or exchange of specimens.

Literature Cited

Atkinson GF. 1897. Some fungi from Alabama. Cornell Univ. Sci. Bull. 3:1-50.

Bauer R, Begerow D, Nagler A, Oberwinkler F. 2001. The Georgefischeriales: a phylogenetic hypothesis. Mycol. Res. 105:416-424.

Beeli M. 1922. Notes mycologiques. Bull. Jard. Bot. État 8:1-22.

Brefeld O. 1883. Botanische Untersuchungen über Hefenpilze. 5. Die Brandpilze I (Ustilagineen). Leipzig, Verlag v. A. Felix, VI + 220 pp. + Pls. I-XIII.

Brefeld O. 1895. Untersuchungen aus dem Gesammtgebiete der Mykologie. XII. Hemibasidii. Brandpilze III. Münster i. W., Commissions-Verlag v. H. Schöningh. IV + 99-236 pp. + Pls. VI-XII.

Bubák F. 1912. Houby České. Díl II. Sněti (Hemibasidii). Arch. Přír. Výzk. Čech. 15(3):1-84.

Canonaco A. 1937. Contributo alla flora micologica dell'Africa Orientale Italiana. Boll. Stud. Inform. Reale Giardino Colon. 14:33-37 + Pl.

Ciferri R.1928. Quarta contribuzione allo studio degli *Ustilaginales*. Ann. Mycol. 26:1-68 + Pl.

Ciferri R, Herter WG. 1932. *Ustilaginales* Uruguayenses; Itinera Herteriana. Bot. Arch. 34:527-540.

Clayton WD, Renvoize SA. 1986. Genera graminum. Grasses of the world. Kew Bulletin Additional Series XIII. London, UK, 389 pp.

Clinton GP. 1902. North American *Ustilagineae*. J. Mycol. 8:128-156.

Clinton GP. 1904. North American *Ustilagineae*. Proc. Boston Soc. Nat. Hist. 31:329-529.

Cornu M. 1883. Sur quelques Ustilaginées nouvelles ou peu connues. Ann. Sci. Nat. Bot., Sér. 6, 15:269-296.

Cunnington J, Vánky K, Shivas RG. 2005. *Lundquistia* is a synonym of *Sporisorium* (Ustilaginomycetes). Mycol. Balcan. 2:95-99.

Dietel P. 1897. Hemibasidii (*Ustilagineae* und *Tilletiineae*). In Engler & Prantl, Die Natürlichen Pflanzenfamilien. I(1)**:2-24.

Doidge EM. 1950. The South African fungi and lichens to the end of 1945. Bothalia 5:1-1045. (*Ustilaginales* pp. 377-393).

Durán R. 1970. Hosts and distribution records of Mexican smut fungi. Mycologia 62:1094-1105.

Durán R. 1972. Aspects of teliospore germination in North American smut fungi. II. Canad. J. Bot. 50:2569-2573 + Pls. I-IV.

Durán R. 1987. *Ustilaginales* of Mexico. Taxonomy, symptomatology, spore germination, and basidial cytology. Washington State University, Pullman, USA, 331 pp.

Ellis JB, Galloway BT. 1890. New species of fungi. J. Mycol. 6:31-33.

Fischer GW. 1953. Manual of the North American smut fungi. New York, Ronald Press Co.

Griffiths DG. 1902. Concerning some West American fungi. Bull. Torrey Bot. Club 29:290-301.

Guo L. 1990. The genera *Sorosporium* and *Sporisorium* in China. Mycosystema 3:67-88.

Guo L, Vánky K, Mordue JE. 1990. The genus *Franzpetrakia* (*Ustilaginales*). Mycosystema 3:57-66.

Hennings P. 1893. Fungi africani, II. Bot. Jahrb. Syst. 17:1-42.

Hennings P. 1895a. Neue und interessante Pilze aus dem Königl. botanischen Museum in Berlin. III. Hedwigia 34:10-13.

Hennings P. 1895b. Fungi goyazenses. Hedwigia 34:88-116.

Hennings P. 1895c. *Ustilagineae*. Brandpilze. In Engler, A. (ed.). Die Pflanzenwelt Ost-Afrikas und der Nachbargebiete. Teil C. Berlin, pp. 48-49.

Hennings P. 1896a. Ustilaginaceen. In Pazschke O., II. Verzeichniss brasilianischer von E. Ule gesammelter Pilze. Hedwigia 35:50-51.

Hennings P. 1896b. Beiträge zur Pilzflora Südamerikas I. Myxomycetes, Phycomycetes, *Ustilagineae* und *Uredineae*. Hedwigia 35:207-262.

Hennings P. 1897. Beiträge zur Pilzflora Südamerikas II. Hedwigia 36:190-246.

Hennings P. 1898. Fungi americani-boreales. Hedwigia 37:267-276.

Hennings P. 1900. Die Gattung *Pericladium* Passer. Hedwigia, Beibl. 39:(75)-(76).

Hennings P. 1904. Fungi amazonici I. Hedwigia 43:154-186.

Herbert DA, Langdon RFN. 1941. Records of Queensland fungi, III. Univ. Queensland Dept. Biol. Pap. 2:1-6.

Hirschhorn E. 1941. Las especies de *Sorosporium* de la flora Argentina. Revista Mus. La Plata, N.S., Secc. Bot. 3:335-354 + Pls. I-IV.

Hirschhorn E. 1986. Las *Ustilaginales* de la flora Argentina. La Plata, CIC, 530 pp.

Holway EWD. 1899. Mexican fungi. II. Bot. Gaz. 28:273-274.

Ingold CT. 1996. Different patterns of teliospore germination in *Ustilago trichophora*. Mycol. Res. 100:418-420.

Jackson HS. 1908. *Sorosporium ellisii* Winter, a composite species. Bull. Torrey Bot. Club 35:147-149.

Jennings HS. 1890. Some parasitic fungi of Texas. Texas Agric. Exp. Sta. Bull. 9:23-29.

Kochman J. 1936. Grzyby Głowniowe Polski. *Ustilaginales* Poloniae. Planta Polonica. 4:1-161.

Lindeberg B. 1959. *Ustilaginales* of Sweden (exclusive of the *Cintractia*s on Caricoideae). Acta Univ. Upsal., Symb. Bot. Upsal. 16(2):1-175.

Ling L. 1949. Taxonomic notes on Asiatic smuts. I. Sydowia 3:123-134.

Ling L. 1950. Studies in the genus *Cintractia*. I. *Cintractia montagnei* and related species. Mycologia 42:503-513.

Ling L. 1951.Taxonomic notes on Asiatic smuts. III. Sydowia 5:40-48.

Ling L. 1953a. Taxonomic notes on the *Ustilaginales*. II. Lloydia 16:180-192.

Ling L. 1953b. Taxonomic notes on the *Ustilaginales*. III. Sydowia 7:151-157.

Magnus P. 1900. J. Bornmüller, Iter Syriacum 1897. Fungi. Weiterer Beitrag zur Kenntnis der Pilze des Orients. Verh. K. K. Zool.-Bot. Ges. Wien 50:432-449 + Pls. II-III.

Maire R. 1917. Champignons nord-africains nouveaux ou peu connus. Bull. Soc. Hist. Nat. Afrique N. 8:134-200.

Maire R, Werner RG. 1937. Fungi Maroccani. Mém. Soc. Sci. Nat. Maroc. 45:1-147.

Massey AB, Zundel GL. 1942. *Sorghastrum*, host of an undescribed smut. Phytopathology 32:544-546.

Mundkur BB. 1944. Some rare and new smuts from India. Indian J. Agric. Sci. 14:49-52.

Opiz PM. 1823-25. Naturalientausch. No. 1-10. Prag. Sechstes Verzeichniss 1924:37-52.

Passerini G. 1875. Funghi raccolti in Abissinia dal Signor O. Beccari, esaminati dal Prof. G. Passerini. Nuovo Giorn. Bot. Ital. 7:180-192.

Pavgi MS, Thirumalachar MJ. 1957. *Franzpetrakia*, a new genus of the *Ustilaginales*. Sydowia, Beih. 1:1-3 + Pl. 1.

Piepenbring M. 1995. Taxonomic studies on *Ustilaginales* from Costa Rica. Mycol. Res. 99:783-788.

Piepenbring M. 1996. Smut fungi (*Ustilaginales* and *Tilletiales*) in Costa Rica. Beih. Nova Hedwigia 113:1-155.

Piepenbring M. 1999. New and poorly known smut fungi in Cuba. Mycol. Res. 103:459-467.

Piepenbring M. 2000. The species of *Cintractia* s. l. (*Ustilaginales*, Basidiomycota). Nova Hedwigia 70:289-372.

Piepenbring M. 2002a. Diversity, taxonomy, and ecology of plant parasitic smut fungi in Bolivia. Ecologia en Bolivia 37:49-58.

Piepenbring M. 2002b. Annotated checklist and key for smut fungi in Colombia. Caldasia 24:103-119.

Piepenbring M. 2003. Smut fungi (Ustilaginomycetes p.p. and *Microbotryales*, Basidiomycota). Flora Neotropica. Monograph 86. The New York Botanical Garden Press, New York.

Piepenbring M, Begerow D, Oberwinkler F. 1999. Molecular sequence data assess the value of morphological characteristics for a phylogenetic classification of species of *Cintractia*. Mycologia 91:485-498.

Pole-Evans IB. 1915. Three fungi collected on the Percy Sladen Memorial Expeditions of 1910-11 and 1912-13. Ann. Bolus Herb. 1:115.

Prasad N, Tyagi RNS. 1961. A new species of *Pericladium* causing stem galls on *Grewia flavescens* Juss. J. Indian Bot. Soc. 40:497-501.

Scholz H, Scholz I. 1988a. Die Brandpilze Deutschlands (*Ustilaginales*). Englera 8:1-691.

Scholz H, Scholz I. 1988b. Taxonomische Erkenntnisse bei der Erarbeitung einer Brandpilzflora von Deutschland (*Ustilaginales*). Nova Hedwigia 47:69-79.

Scholz H, Scholz I. 2000. Die Brandpilze Deutschlands (*Ustilaginales*), Nachtrag. Verh. Bot. Vereins Berlin Brandenburg 133:343-398.

Scholz H, Scholz I. 2004. Die Brandpilze Deutschlands, 2. Nachtrag. Verh. Bot. Vereins Berlin Brandenburg 137:441-487.

Schwarzman SR. 1960. Golovnevye griby. (Smut fungi; in Russian). Flora sporovych rastenij Kazachstana 2. Alma Ata.

Spegazzini C. 1899. Fungi argentini novi vel critici. Anales Mus. Nac. Buenos Aires, Ser. 2, 6:81-367 + 2 Pls.

Sydow H. 1924. Notizen über Ustilagineen. Ann. Mycol. 22:277-291.

Sydow H. 1937. Neue oder bemerkenswerte australische Micromyceten. I. Ann. Mycol. 35:22-49.

Sydow H. 1939. Novae fungorum species. XXVII. Ann. Mycol. 37:197-253.

Sydow H, Sydow P. 1912. Novae fungorum species. VIII. Ann. Mycol. 10:405-410.

Sydow H, Sydow P, Butler EJ. 1912. Fungi Indiae orientalis. Pars IV. Ann. Mycol. 10:243-280.

Thirumalachar MJ. 1946. Nuclear cycle and life history of a new species of *Doassansia* (*Ustilaginales*). Lloydia 9:24-30.

Thirumalachar MJ. 1947. Species of the genera *Doassansia, Doassansiopsis*, and *Burrillia* in India. Mycologia 39:602-611.

Thirumalachar MJ, Neergaard, P. 1977(1978). Studies on the genus *Tolyposporium*. *Tolyposporidium* gen. nov. Friesia 11:177-192.

Thirumalachar MJ, Pavgi MS. 1967(1968). Notes on some Indian *Ustilagineae.* IX. Sydowia 20:21-27 + Pl. I.

Thirumalachar MJ, Pavgi MS. 1968(1969). Notes on some Indian *Ustilagineae.* X. Sydowia 22:250-253 + Pls. XV-XVI.

Tracy SM, Earle FS. 1895. New species of parasitic fungi. Bull. Torrey Bot. Club 22:174-179.

Tulasne L-R, Tulasne C. 1847. Mémoire sur les Ustilaginées comparées aux Urédinées. Ann. Sci. Nat. Bot., Sér. 3, 7:12-127 + Pls. 2-7.

Vánky K. 1983. Ten new species of *Ustilaginales.* Mycotaxon 18:319-336.

Vánky K. 1985. Carpathian *Ustilaginales.* Acta Univ. Upsal., Symb. Bot. Upsal. 24(2):1-309.

Vánky K. 1990. Taxonomical studies on *Ustilaginales.* VI. Mycotaxon 38:267-278.

Vánky K. 1993. Taxonomical studies on *Ustilaginales.* X. Mycotaxon 48:27-44.

Vánky K. 1996. Taxonomical studies on *Ustilaginales.* XIV. Mycotaxon 59:89-113.

Vánky K. 1997a.Taxonomical studies on *Ustilaginales.* XVI. Mycotaxon 65:133-158.

Vánky K. 1997b. Taxonomical studies on *Ustilaginales.* XVII. Mycotaxon 65:159-182.

Vánky K. 2000. Taxonomical studies on *Ustilaginales.* XX. Mycotaxon 74:161-215.

Vánky K. 2001. Taxonomical studies on *Ustilaginales.* XXI. Mycotaxon 78:265-326.

Vánky K. 2002a. Taxonomical studies on *Ustilaginales.* XXII. Mycotaxon 81:367-430.

Vánky K. 2002b. Illustrated Genera of Smut Fungi. 2nd. Ed. APS Press, 238 pp.

Vánky K. 2003. Taxonomical studies on *Ustilaginales.* XXIII. Mycotaxon 85:1-65.

Vánky K. 2004a. Taxonomic studies on Ustilaginomycetes - 24. Mycotaxon 89:55-118.

Vánky K. 2004b. The smut fungi (Ustilaginomycetes) of *Bothriochloa, Capillipedium* and *Dichanthium* (*Poaceae*). Fungal Diversity 15:221-246.

Vánky K. 2004c. New smut fungi (Ustilaginomycetes) from Mexico, and the genus *Lundquistia.* Fungal Diversity 17:159-190.

Vánky K. 2004d. *Pilocintractia* gen. nov. (Ustilaginomycetes). Mycol. Balcan. 1:169-174.

Vánky K. 2005a. Taxonomic studies on Ustilaginomycetes - 25. Mycotaxon 91:217-272.

Vánky K. 2005b. The smut fungi (Ustilaginomycetes) of *Chrysopogon* (Poaceae). Fungal Diversity 18:177-187.

Vánky K, Deml G, Oberwinkler F. 1988. The smut fungi of *Hyparrhenia hirta* (Gramineae). J. Phytopathology 121:181-191.

Vánky K, Shivas RG. 2001. New smut fungi (Ustilaginomycetes) from Australia. Fungal Diversity 7:145-174. note). Rev. Pathol. Vég. Entomol. Agric. France 37:167-178.

Vánky K, Shivas RG. 2005. (1655) Proposal to conserve the name of *Ustilago scitaminea* against *Ustilago amadelpha* (Fungi, Ustilaginomycetes, *Ustilaginaceae*). Taxon 54:180.

Viennot-Bourgin G. 1958. *Ustilaginales* nouvelles de Guinée française. (Deuxième note). Rev. Pathol. Vég. Entomol. Agric. France 37:167-178.

Walker J, Shivas RG. 1998. Two species of *Sporisorium* on *Chionachne* in tropical Australia, the first Australian record of *Ustilago brunkii* and its transfer to *Jamesdicksonia*. Mycol. Res. 102:1203-1214.

Winter G. 1883a. Fungi nonnulli novi. Hedwigia 22:1-3.

Winter G. 1883b. New North American fungi. Bull. Torrey Bot. Club 10:7.

Zambettakis C. 1979(1980). Recherches sur les *Ustilaginales*. (Supplément sur les espèces africaines). Bull. Soc. Mycol. France 95:393-443.

Zundel GL. 1930. Monographic studies on the *Ustilaginales* attacking *Andropogon*. Mycologia 22:125-158.

Zundel GL. 1931. Notes on new species of *Ustilaginales*. Mycologia 23:296-299.

Zundel GL. 1933. New and rare North and South American *Ustilaginales*. Mycologia 25:349-355.

Zundel GL. 1938. The *Ustilaginales* of South Africa. Bothalia 3:283-330.

Zundel GL. 1939. Studies on the *Ustilaginales* of the world. Mycologia 31:572-589.

Zundel GL. 1943. Notes on the *Ustilaginales* of the world. III. Mycologia 35:164-184.

Zundel GL. 1951. Notes on the *Ustilaginales* of the world. V. Mycologia 43:267-270.

Zundel GL. 1953. The *Ustilaginales* of the World. Pennsylvania State Coll. School Agric. Dept. Bot. Contrib. 176:XI + 1-410.

MYCOTAXON

Volume 95, pp. 67–69 January–March 2006

Kalmusia amphiloga comb. nov. on *Bambusa*

Ove E. Eriksson

ove.eriksson@emg.umu.se
Department of Ecology and Environmental Science, Umeå University
SE 90187 Umeå, Sweden

Abstract—*Leptosphaeria amphiloga* is transferred to the genus *Kalmusia*. It differs from *K. scabrispora*, another bambusicolous species, in smaller ascomata, asci and ascospores.

Key words—bambusicolous, pyrenomycete, morphology

Introduction

Tanaka et al. (2005: 110) recently transferred *Leptosphaeria scabrispora* Teng to the genus *Kalmusia* Niessl. That species occurs on *Phyllostachys* and is certainly closely related to another bambusicolous species, *Leptosphaeria amphiloga*, which is transferred here to *Kalmusia*.

Kalmusia amphiloga (Petr.) O. E. Erikss., **comb. nov.** **Fig. 1ab**

Basionym: *Leptosphaeria amphiloga* Petr., in Sydow & Petrak, *Ann. Mycol.* 29: 202 (1931).

The presence of this fungus on old bamboo culms is indicated only by the epidermis being raised by the ascomata over small scattered areas, c. 10-40 x 10 mm, up to c. 150 µm high. There are several densely aggregated ascomata within each elevated area, which may have several, narrow, longitudinal cracks, but the ascomata are not visible, only the minute, ascomal pores may be visible.

Pseudostroma subepidermal and visible only if the epidermis is removed, forming a thin, dark brown layer in small, scattered, circular areas on the wood; hyphae intermingled with the uppermost cell layer of the wood, and not distinctly visible as discrete hyphae, irregularly dark reddish brown, turning greenish brown with NaClO or KOH.

Ascomata subepidermal, several densely aggregated in one layer on each pseudostroma (Fig. 1a), flattened subglobose or of more irregular shape from mutual pressure, c. 200-300 µm across; wall c. 10-12 µm thick, pale brown to hyaline.

Hamathecium of numerous, parallel hyphae (pseudoparaphyses?) between and above asci, and here with few branches and anastomoses; cells hyaline, rather thick-walled, most of them c. 25-35 µm long and c. 2 µm wide, with most of the protoplasm (1.5-3 µm) near the septa, slightly constricted at some septa; in the periphery hyphae with more anastomoses and shorter cells.

Asci c. 85-100 x 14-17 µm, clavate, bitunicate; ectotunica thin, endotunica thin (also in asci with broken ectotunica), with a small ocular chamber, but no ring structures; with 8 spores, the two lowermost overlapping uniseriate, the others biseriate.

Ascospores (Fig. 1b) 26-31 x 8-9 µm, fusiform, lower hemispore often with more rounded end, inequilateral, (2-)3-5-septate, very seldom with a longitudinal septum in one of the segments, pale brown, with dark brown, coarse verrucae, and a hyaline sheath (width could not be measured safely, as the material is old and the ascospores do not readily leave the asci).

Anamorph not seen.

Habitat: old culms of *Bambusa* sp.

Known distribution: Asia (Philippines).

> **Material studied:** Philippines, Luzon, Pampanga Prov., Stotsenberg, on *Bambusa* sp., iii.1923, leg. Mary Strong Clemens, n. 1632 (**W** 11891, holotype).

Discussion

Ascomata of *Leptosphaeria* species have walls with scleroplectenchymatous cells (Holm 1957). *Leptosphaeria amphiloga* differs in that respect from typical members of the genus. It resembles very much another bambusicolous species, *Leptosphaeria scabrispora*, which recently was transferred to *Kalmusia* by Tanaka et al. (2005: 110), and *L. amphiloga* is now placed in the same genus. *Kalmusia scabrispora* (Teng) Tanaka et al. and *K. amphiloga* both have ascomata that are densely aggregated under the epidermis in elevated areas of bark on bamboo stems. The ascomata, asci and ascospores of the former species are larger. In *K. scabrispora,* the ascomata measure 250-400 µm, the asci 124-153(-160) x (15.5-) 17-21 µm, and the ascospores (29-)31-40.5 x (7-)8-10 µm (Tanaka et al., l.c.), whereas in *K. amphiloga,* the ascomata measure c. 200-300 µm, the asci c. 85-100 x 14-17 µm, and the ascospores c. 26-31 x 8-9 µm.

Kalmusia is currently accommodated in the *Montagnulaceae* (Eriksson 2006: 7), but there are no sequences in GenBank from any member of the genus, and its position is uncertain.

Acknowledgements

I am grateful to the Curator of W for the loan of the type material of *Leptosphaeria amphiloga,* to Dr Jingzhu Pearl Yue (San Francisco) for preparing a drawing of a bamboo twig with immersed ascomata of the species, and to the reviewers Prof. L. Holm and Prof. N. Lundqvist (Uppsala) for examining this paper.

Literature Cited

Eriksson O.E. (ed.). 2006. Outline of the Ascomycota – 2006. Myconet 12: 1-82.

Holm L. 1957. Études taxonomiques sur les Pléosporacées. - Symbolae Botanicae Upsalienses 14(3): 1-188.

Sydow H. & Petrak F. 1931. Micromycetes philippinenses. Series secunda. Ann. Mycol. 29: 145-279.

Tanaka K, Harada Y, Barr ME 2005. Bambusicolous fungi in Japan (3): a new combination, *Kalmusia scabrispora.* Mycoscience 46: 110-113.

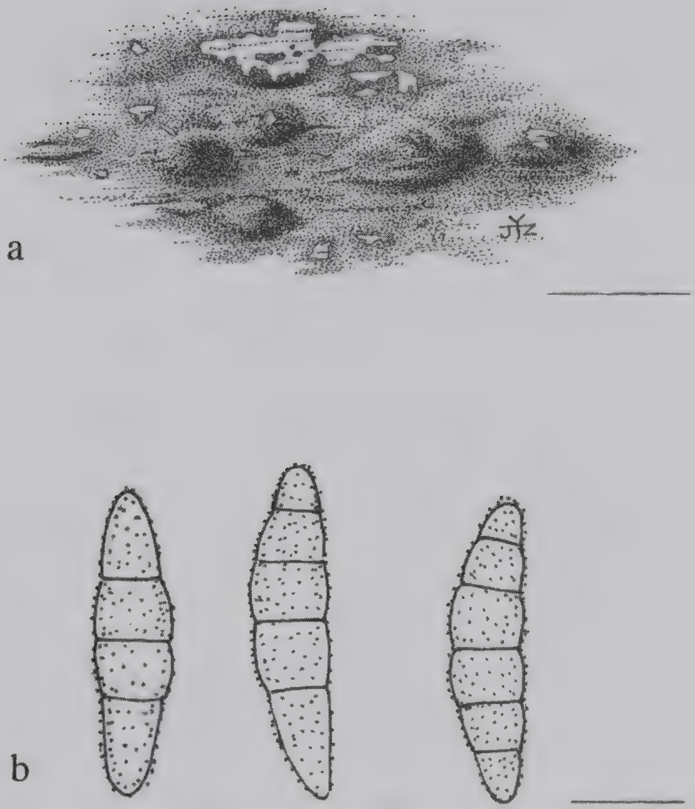


Fig. 1 a-b. *Kalmusia amphiloga*. a. Bark with bulges, each containing several ascomata (del. J.-Z. Yue). b. ascospores. Scale: a. 1 mm, b. 10 µm. – Material: a-b. W11891.

MYCOTAXON

Volume 95, pp. 71–79 *January–March 2006*

A new species and new records of *Rhytismatales* from Taiwan

Cheng-Lin Hou[1] Roland Kirschner[2] Chee-Jen Chen[3]

houchenglincn@yahoo.com
[1]College of Life Science, Capital Normal University
Beijing 100037, China

kirschner@em.uni-frankfurt.de
[2]Botanisches Institut, Johann Wolfgang Goethe-Universität
Frankfurt am Main, 60054 Frankfurt/M., Germany

C5200999@mail.stut.edu.tw
[3]Department of Biotechnology, Southern Taiwan University of Technology
1 Nan-Tai street, Yung-kang City, Tainan County, Taiwan 71043

Abstract—Of nine names of the *Rhytismatales* reported for Taiwan, only five, *Coccomyces foliicola, Lophodermium mangiferae, L. pinastri, Rhytisma placenta,* and *Vladracula annuliformis,* are retained. The other four are either invalid or incorrect. Recently, additional specimens were collected and identified. They include a new species, *Coccomyces taiwanensis* on fallen twigs of an unknown angiosperm host, and three species reported for the first time for Taiwan, *Coccomyces multangularis* on *Machilus thunbergii, Lophodermium conigenum* on *Pinus* sp. and *Lophodermium petrakii* on *Cunninghamia lanceolata.*

Key words—Ascomycota, *Rhytismataceae*, taxonomy

Introduction

Until now, nine names of the *Rhytismatales* (Ascomycota) have been reported for Taiwan. Some of them, however, are invalid or incorrect. *Lophodermium rottboelliae* Sawada ('*rottobaelliae*'), *Rhytisma ilicis* Sawada, and *R. rhododendri-oldhamii* Sawada are invalidly published since Sawada did not provide Latin diagnoses for them (Hou 2004, Sawada 1943, 1944, 1959), and *Coccomyces mussaendae* Sawada is a synonym of *Biostictis tjibodensis* (Racib.) Sherwood (*Ostropales*) (Sherwood 1980).

Therefore, there are only five correctly applied names of the *Rhytismatales* in Taiwan, i.e. *C. foliicola* (Dennis & Spooner) Sherwood, *L. mangiferae* Koord., *L. pinastri* (Schrad.) Chevall., *R. placenta* Berk. & Broome, and *Vladracula annuliformis* (Syd. et al.) P.F. Cannon et al. (Sawada 1959, Sivanesan & Hsieh 1989, Li & Hsieh 1991, Chen & Hsieh 1994, Wu & Wang 2000). The present study, which is based on specimens recently collected by the second and third authors from Taiwan, includes one new species and three species previously unknown from Taiwan.

---

* *Author for correspondence*

Materials and methods

Sections of different thickness of ascomata were made by hand using a razor blade. Microscopic preparations were made in water, Melzer's reagent, 5% KOH, or 0.1% (w/v) cotton blue in lactic acid. For observation of ascomatal outlines in vertical section, sections were mounted in lactic acid or cotton blue with pretreatment in water. Gelatinous sheaths surrounding ascospores and paraphyses were observed in water or cotton blue in lactic acid. Ascospore contents are drawn based on observations in water. Measurements were made using material mounted in 5% KOH or Melzer's reagent and from 20 ascospores and asci for each specimen.

Taxonomy

Coccomyces multangularis Y.R. Lin & Z.Z. Li Mycosystema 20: 1, 2001

FIGURES 1-5

> Holotype. On *Litsea coreana* var. *sinensis* (Allen) Yang & P. H. Huang, China, Anhui, Huangshan, Zuishi, 12 VIII 1995, Y. R. Lin et al. L1621a (AAUF 67729a).

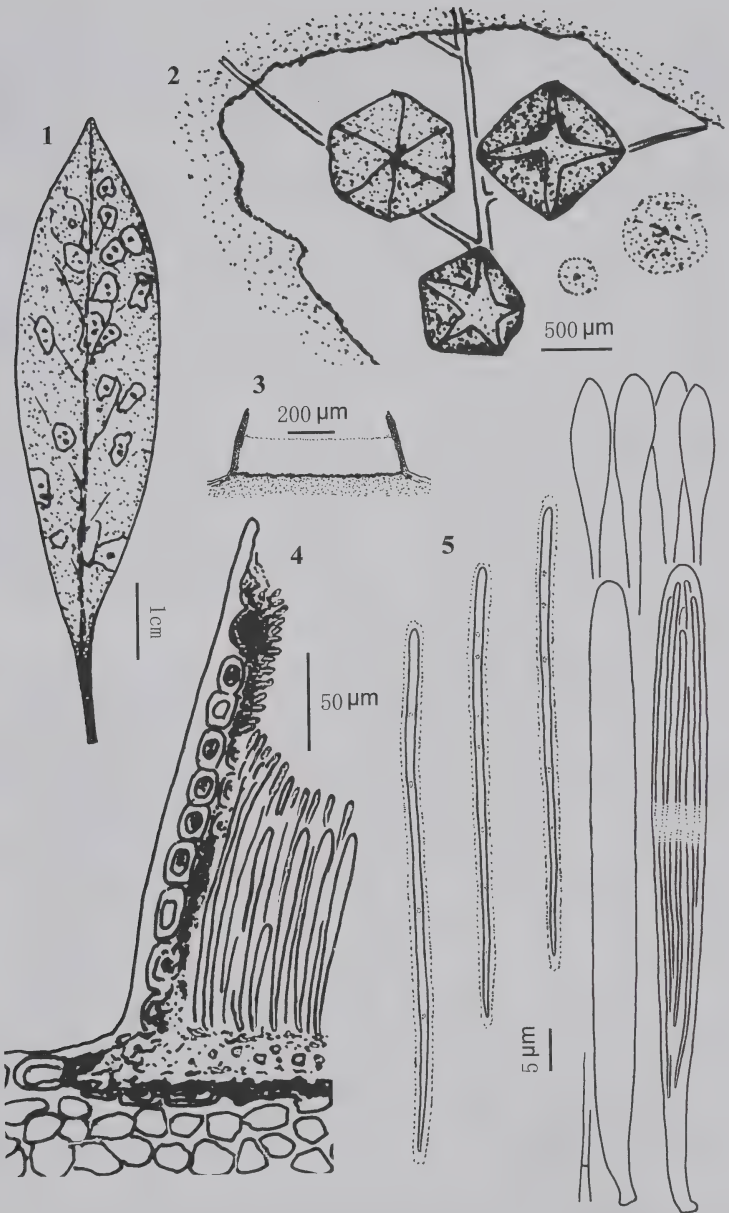
Ascomata developing on both sides of fallen leaves, in small, bleached or pale brown lesions, angular, 4-6-sided, occasionally triangular, round when young, (500-)600-1100 μm diam., black, shiny on the upper side of the leaf, and less shiny on the lower side of the leaf, strongly raising above the surface of the host at maturity, with obvious, preformed lines of dehiscence, lips absent, opening by 4-6 teeth. In median vertical section, ascomata intraepidermal at ascomatal edges, subepidermal to intrahypodermal near the middle part of covering stroma, and intraepidermal to cuticular near the opening, 220-320 μm deep, covering stroma poorly developed, 20-35 μm thick, consisting of textura angularis and hyphae with thick-walled, dark brown fungal cells. Epidermal and remained hypodermal cells in covering stroma filled with dark brown fungal cells of different size. Periphyses short cylindrical, hyaline, thin-walled, 3-5 × 1-2 μm. Basal stroma medium developed, flat, composed of two layers of dark brown, thick-walled angular cells, 10-13 μm thick. Excipulum present, 20-30 μm wide, arising from the marginal paraphyses. Excipular elements similar to paraphyses. Subhymenium consisting of textura intricata, 15-20 μm thick, with many crystals of different shape and size. Paraphyses 100-120 × 1.5-2 μm, filiform, aseptate, not branched, swollen up to 4-5 μm diam. at the apex. Asci ripening sequentially, 80-110 × 4-5 μm, cylindrical, thin-walled, J–, without circumapical thickening at the apex, 8-spored. Ascospores filiform, 40-65 × 0.8 μm, rounded at the apex and slightly tapering towards the base, hyaline, aseptate, multiguttulate, with a thin gelatinous sheath.

Structures resembling conidiomata of *Rhytismatales* in pale areas near ascomata, pale brown to concolorous with the host tissue, round, 180-240 μm diam., opening by an ostiole. In vertical section, conidiomata epidermal. Conidiogenous cells and conidia not seen.

> Specimen examined: TAIWAN, Taipei, Yangmingshan, alt. ca. 900 m, on fallen leaves of *Machilus thunbergii* Sieb. & Zucc. (*Lauraceae*), 27 VIII 2004, Roland Kirschner (TNM).

Known distribution: Anhui (Lin et al. 2001) and Taiwan.

Known Host species: *Litsea coreana* var. *sinensis*, *Machilus thunbergii*. (Lin et al. 2001).



Figs. 1-5. *Coccomyces multangularis* on fallen leaves of *Machilus thunbergii*. 1. A leaf bearing ascomata. 2. Ascomata as seen under a dissecting microscope. 3. Ascoma in vertical section. 4. Detail of an ascoma in vertical section. 5. Paraphyses, a mature ascus with ascospores, an ascus after the liberation of the ascospores, and liberated ascospores with gelatinous sheaths.

Notes: The morphological features, ecology and host of the specimen are very similar to the type specimen of *Coccomyces multangularis* described by Lin et al. (2001). Depth of ascomata inserting the host of *C. multangularis* is similar to that of *C. mucronatus* Korf & W. Y. Zhuang on fallen leaves of *Fagaceae* (Korf & Zhuang 1985). *C. mucronatus* is easily distinguished from *C. multangularis* by its mucronate tip of the paraphyses.

Coccomyces taiwanensis C.L. Hou, R. Kirschner & Chee J. Chen, **sp. nov.**

FIGURES **6-11**

> *Ascomata (1200-)1500 × 2500 µm, nigra, elliptica, intraepidermalia; paraphyses filiformes; asci (150-)180-220 × 8-10 µm, clavati; ascosporae 100-150 × 1-1.5 µm, filiformes.*
>
> *Etymology*: referring to the place where the specimen was collected.
>
> HOLOTYPE: On twigs of unknown angiosperm host, TAIWAN, Yilan, Taipingshan, alt. ca. 1500 m, 24 V 2000, R. Kirschner & Chee. J. Chen 662 (TNM).

Ascomata developing on fallen twigs, not associated with bleached areas. Ascomata irregularly rounded or slightly triangular, occasionally elliptical, (1200-)1500 × 2500 µm diam., black, strongly raising above the surface of the host at maturity, without preformed lines of dehiscence, lips absent, opening by irregular splits. In median vertical section, ascomata deep in host tissue, 300-450 µm deep, covering stroma up to 75-120 µm thick near the centre of the ascomata (not including host tissue), slightly thinner towards the edges, extending to the basal stroma, consisting of an outer layer of host tissue and an inner layer of black textura angularis and short hyphae.

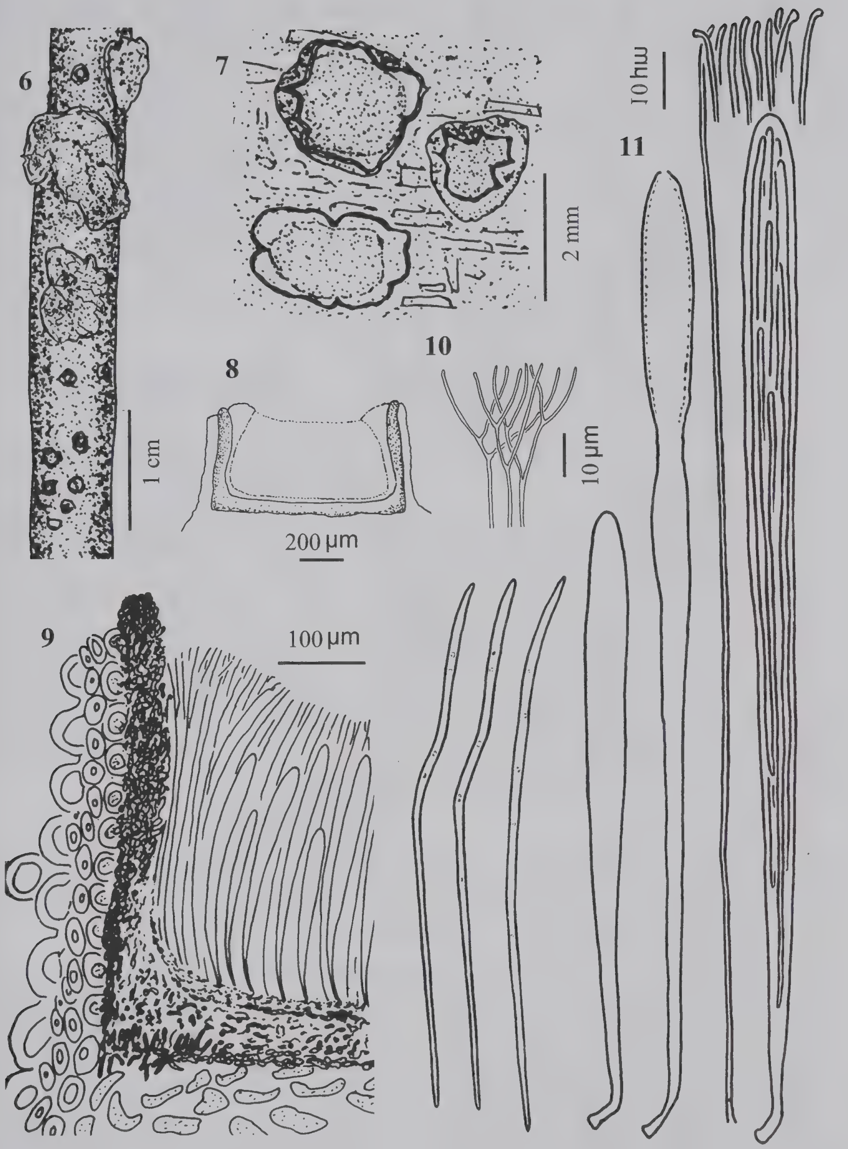
Periphysoids absent. Basal stroma flat, 30-50 µm thick, composed of an outer layer of dark brown short hyphae or textura angularis and an inner layer of pale brown to hyaline short hyphae. *Excipulum* well developed, 80-120 µm wide, arising from the marginal paraphyses. Excipular elements branching two times near the apices. Subhymenium consisting of textura intricate, 10-15 µm thick. Paraphyses 200-250 × 1 µm, filiform, unbranched, simple, slightly hooked at the apex. Asci ripening sequentially, (150-)180-220 × 8-10 µm, long cylindrical-clavate, with a conspicuous stalk, thin-walled, J–, without circumapical thickening, 8-spored. Ascospores fasciculate, 100-150 × 1-1.5 µm, filiform, tapering at the both ends, hyaline, aseptate, gelatinous sheaths not seen.

Conidiomata and zone lines not observed.

Known distribution: Only from the type locality.

Habitat: *C. taiwanensis* was collected from twigs in litter.

Notes: Microscopical investigations of wood anatomy of the substrate of *C. taiwanensis* revealed the presence of pitted vessels, indicating that the host belongs to angiosperm. *Coccomyces taiwanensis* is ecologically and macroscopically similar to some *Coccomyces* species on bark, such as *C. boydii* A. L. Sm. on *Myrica gale* L., *C. juniperi* (P. Karst.) P. Karst. on *Juniperus* spp., and *C. strobi* J. Reid & Cain on *Pinus* spp. (Sherwood 1980). However, *C. taiwanensis* has a very well developed excipulum and excipular elements branching two times near the apices, while *C. boydii*, *C. juniperi*, and *C. strobi* do not have an excipulum (Sherwood 1980). In addition, shape and size of ascospores as well as lacking of gelatinous sheaths surrounding the ascospores of the new taxon are quite different from those of the species mentioned above (Sherwood 1980). The shape of the



Figs. 6-11. *Coccomyces taiwanensis* on twigs of unknown angiosperm host. 6. A twig bearing ascomata. 7. Ascomata as seen under a dissecting microscope. 8. Ascoma in vertical section. 9. Detail of an ascoma in vertical section. 10. Detail of the top of excipular elements. 11. Paraphyses, a young ascus, a mature ascus with ascospores, an ascus after the liberation of the ascospores, and liberated ascospores.

released ascospores of *C. taiwanensis* is reminiscent of the species of *Lophodermium unciniae* and *L. brunneolum* (Johnston 1994) where the ascospore has a slight bend along its length. For the new taxon, however, no a small gelatinous appendage at the point where the ascospore is bent is observed.

Lophodermium conigenum (Brunaud) Hilitzer, Věd. Spisy čsl. Akad. zeměd. 3: 76, 1929.

Lophodermium pinastri forma *conigena* Brunaud, 1888; *Lophodermina conigena* (Brunaud) Tehon, 1935. Neotype. On *Pinus sylvestris* L., Scotland, Glentanar, Aberdeenshire, 5 IX 1975, C. S. Millar s. n. IMI 231805.

For description and figures of *L. conigenum* see Minter (1981, 1985).

Specimen examined: TAIWAN, Nantou, Huisunlinchang, ca. 800 m, on fallen needles of *Pinus* sp., 11 VIII 2004, R. Kirschner & C. J. Chen 2015 (TNM).

Known distribution: Europe, North America, Asia, Australia, Africa (Minter 1981).

Host species: *Pinus* sp. (*Pinaceae*). For further host species see Minter (1981).

Habitat: *L. conigenum* was collected from needles that were still attached to twigs or fallen on the ground.

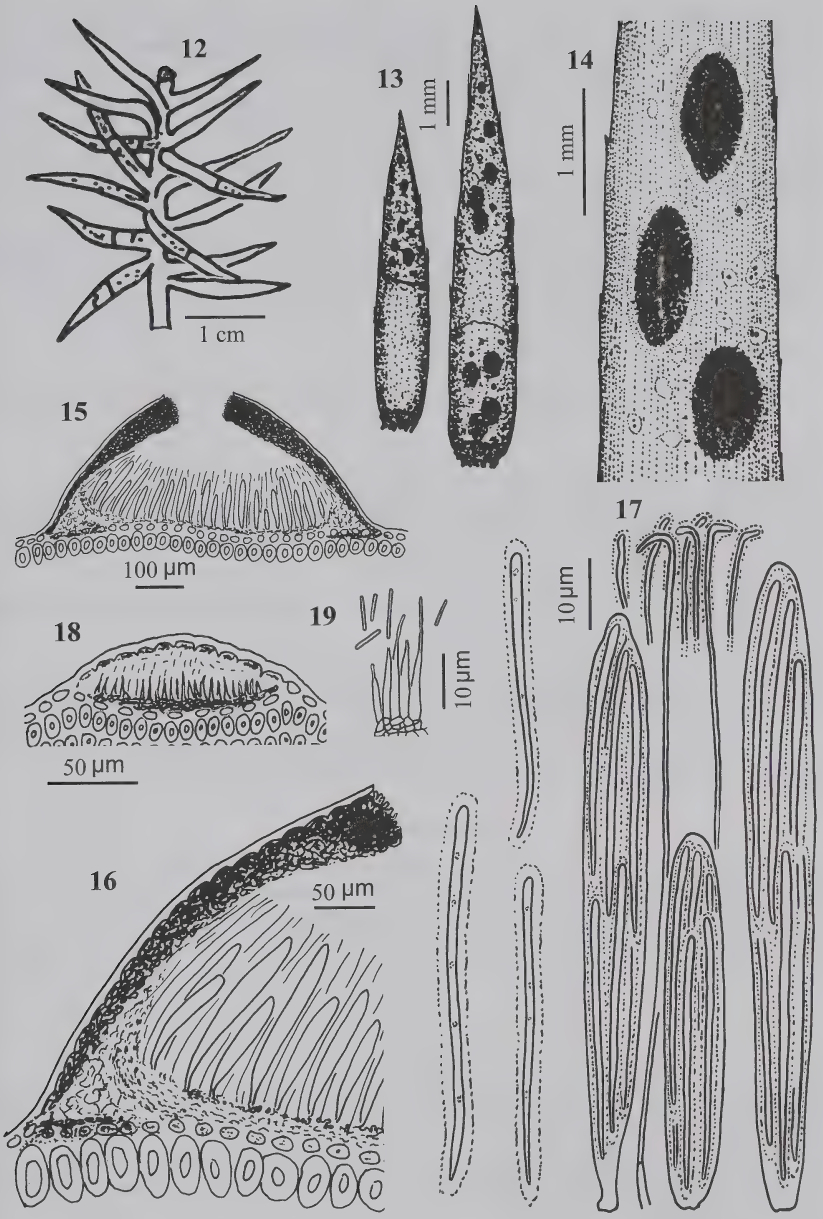
Notes: *Lophodermium conigenum* is morphologically similar to *L. australe* Dearn., and Minter (1985) considered that they might be conspecific. The specimen we checked matches the description of *L. conigenum* by Minter (1981) except that the dimensions of the ascomata and ascospores are somewhat smaller. Our collection differs from Minter's concept of *L. australe* by its wider ascomata which partly insert epidermal cells of host.

The nucleotide sequences of the internal transcribed spacer (ITS) show that specimens identified as both these species are genetically very similar (Ortiz-Garcia et al. 2003). However, unpublished ITS sequence data of the first author shows that there may be several genetically distinct but morphologically very similar *L. conigenum*-like species. DNA sequences were not obtained for the Taiwanese specimen.

Lophodermium petrakii Durrieu, Sydowia Beih. 1: 355, 1957. FIGURES **12-19**

Type. On *Cunninghamia lanceolata* (Lamb.) Hook., France, Arboretum Henri Gaussen du Laboratoire forestier de Toulouse, á Jouéou prés Luchon, IX 1955, Herbier du Laboratoire Botanique Appliquée, Toulouse (TLA 1822).

Ascomata on both sides of needles, scattered, sometimes two ascomata confluent. In surface view ascomata dark brown, shiny, perimeter line inconspicuous or absent, elliptical, 700-1100 × 420-580 μm, slightly raising above the surface of the substrate, opening by a single longitudinal split. Lips inconspicuous. In median vertical section ascomata subcuticular, 220-280 μm deep. Covering stroma up to 40-65 μm thick near the centre of the ascomata, thinner towards the edges, not extending to or slightly to the basal stroma, consisting of an outer layer of host cuticle, a layer of textura angularis but textura epidermoidea with thick-walled, dark brown cells near the inner part of the covering layer. A triangular space between the covering stroma and the basal stroma filled with thin-walled, hyaline cells. Lip cells sparse, disappearing at maturity. Basal stroma poorly developed or absent, the epidermal cells underneath basal stroma tinted. Subhymenium 8-15 μm thick, composed of textura intricata. Paraphyses 120-150 ×


12
1 cm
13
1 mm
14
1 mm
15
100 µm
17
10 µm
18
50 µm
19
10 µm
16
50 µm

Figs. 12-19. *Lophodermium petrakii* on *Cunninghamia lanceolata*. 12, 13. Needles bearing ascomata. 14. Ascomata as seen under a dissecting microscope. 15. Ascoma in vertical section. 16. Detail of an ascoma in vertical section. 17. Paraphyses, mature asci with ascospores, and liberated ascospores with gelatinous sheaths. 18. Conidioma in vertical section. 19. Conidiogenous cells and conidia.

2-3 µm, filiform, septate, not branched, slightly swollen at the apex, the paraphyses covered by gelatinous sheaths. Asci ripening sequentially, (60-)80-140 × 10-13 µm, cylindrical, thin-walled, sometimes rostrate at the apex at maturity, J-, without circumapical thickening. Ascospores 50-75 × 1.5-2 µm, filiform, slightly tapering near the base, hyaline, aseptate, with a 1-2 µm thick gelatinous sheath.

Conidiomata on both sides of needles. In surface view conidiomata 100-250 µm diam., elliptical or slightly irregular, concolorous with the substrate or slightly brown, dark brown when old, opening by one to several ostioles. In vertical section conidiomata subcuticular, 23-35 µm deep, upper layer composed of host cuticle and undistinguishable fungal tissue. Basal layer poorly developed. Conidiogenous cells 8-15 × 1-2 µm, cylindrical, tapering towards the tip. Conidia 4-7 × 0.8-1 µm, bacilliform, hyaline.

Zone lines present, frequent, black.

> Specimen examined: TAIWAN, alt. ca. 400 m, 20 III 2001, R. Kirschner (s. n.) (AAUF), TAIWAN, Nantou, Huisunlinchang, ca. 800 m, 12 VIII 2004, R. Kirschner & C. J. Chen 2053 (TNM).

Host species: *Cunninghamia lanceolata* (Lamb.) Hook. (*Taxodiaceae*).

Known distribution: Europe (Durrieu 1957), Anhui, Jiangxi, Sichuan, Guizhou, Jiangsu, Hunan, Fujian, Guangxi, Shandong, Henan, Shanxi (Hou 2000, Lin et al. 1993), Taiwan.

Habitat: *L. petrakii* was collected from needles that were still attached to living and dead twig and shoot.

Notes: *Lophodermium petrakii* was often incorrectly described as *L. pinastri* or *L. uncinatum* Darker in China (Hou 2000, Teng 1996). However, *L. pinastri* only occurs on *Pinus* spp. (Minter 1981) and *L. uncinatum* is only known on *Abies* spp. in North America. They are morphologically quite different from *L. petrakii*.

Acknowledgements

We are grateful to Dr. W.Y. Zhuang and Dr. P.R Johnston for critically reading the manuscript, to T. Trampe for assisting in the wood anatomy of the substrate of *C. taiwanensis,* and to Dr. Y. Z. Wang for providing literature on ascomycetes from Taiwan. The study was supported by the National Science Council (NSC 93-2745-B-218-001-URD) of Taiwan, Deutscher Akademischer Austauschdienst of Germany (DAAD), and the National Natural Science Foundation of China (NSFC).

Literature Cited

Chen CY, Hsieh WH. 1994. Some new ascomycetous fungi for Taiwan. Trans. Mycol. Soc. R. O. C. 9: 39-54.

Durrieu G. 1957. Deux *Hypodermataceae* nouvelles sur conifers. Sydowia Beih. 1: 355-358.

Hou CL. 2000. The pathogenic fungi of *Rhytismataceae* on Chinese fir. Forest Pest and Disease. 19: 3-5. (in Chinese)

Hou CL. 2004. *Rhytismatales* (Ascomycota) in China-Ecology, Morphology, and Systematics [Dissertation]. Frankfurt am Main: J. W. Goethe-Universität Frankfurt, 2004. 279 pp.

Korf RP, Zhuang, WY. 1985. Some new species and new records of discomycetes in China. Mycotaxon 22: 483-514.

Johnston PR. 1994. Ascospore sheaths of some *Coccomyces*, *Hypoderma*, and *Lophodermium* species (*Rhytismataceae*). Mycotaxon 52: 221-239.

Li LT, Hsieh WH. 1991. Two new species and 13 new records of ascomycetes from Taiwan. Trans. mycol. Soc. R. O. C. 6: 57-71.

Lin YR, Tang YP, Liu HY. 1993. Some members of the *Rhytismataceae* on conifers in the southern part of China. Acta Mycol. Sin. 12: 93-98. (in Chinese)

Lin YR, Li ZZ, Xu ZS, Wang JR, Yu SM. 2001. Studies on the genus *Coccomyces* from China IV. Mycosystema 20: 1-7. (in Chinese)

Minter DW 1981. *Lophodermium* on pines. Mycol. Pap. 147: 1-54.

Minter DW. 1985. Some members of the *Rhytismataceae* (Ascomycetes) on conifer needles from Central and North America. Pp. 71-106. In Peterson, G. W. Recent research on conifer needles diseases conference proceedings. Gen. Techn. Rep., WO-50, U. S. Forest Serv.

Ortiz-García S, Gernandt DS, Stone JK, Johnston PR, Chapela IH, Salas-Lizana R, Alvarez-Buylla ER. 2003. Phylogenetics of *Lophodermium* from pines. Mycologia 95: 846-859.

Sawada K. 1943. Descriptive catalogue of the Formosan fungi. Part VIII. Rep. Dept. Agric. Gov. Res. Inst. Formosa 85: 1-130, in: Anonymous. 1999. List of the Fungi in Taiwan. Council of Agriculture, Executive Yuan, Taipei, 289 pp.

Sawada K. 1944. Descriptive Catalogue of Formosan Fungi. Part X. Rep. Dept. Agric. Gov. Res. Inst. Formosa 87: 1-97, in: Anonymous. 1999. List of the Fungi in Taiwan. Council of Agriculture, Executive Yuan, Taipei, 289 pp.

Sawada K. 1959. Descriptive Catalogue of Taiwan (Formosan) Fungi. XI. College of Agriculture, National Taiwan University, Taipei, Taiwan: 1-268 + plates I-XII.

Sherwood MA. 1980. Taxonomic studies in *Phacidiales*: the genus *Coccomyces* (*Rhytismataceae*). Occ. Pap. Farlow Herb. Crypt. Bot. 15: 1-120.

Sivanesan A, Hsieh WH. 1989. New species and records of ascomycetes from Taiwan. Mycol. Res. 93: 340-351.

Teng SC. 1996. Fungi of China. Mycotaxon, Ltd., Ithaca, N.Y. 586 pp.

Wu ML, Wang YZ. 2000. Mycological resources of saprophytic ascomycetes in Fushan Forest. Fung. Sci. 15: 1-14.

MYCOTAXON

Volume 95, pp. 81–90 January–March 2006

Macromycetes of Pinacate and Great Altar Desert biosphere reserve, Sonora, Mexico

Martín Esqueda[1*], Martha Coronado[2], Alfonso Sánchez[1],
Evangelina Pérez-Silva[3] & Teófilo Herrera[3]

*esqueda@cascabel.ciad.mx
[1]Centro de Investigación en Alimentación y Desarrollo
A.C. Apartado Postal 1735, Hermosillo 83000, Sonora. México
[2]Centro de Estudios Superiores del Estado de Sonora
Apartado Postal 11, Admón. 11, Hermosillo 83000, Sonora. México
[3]Laboratorio de Micología, Instituto de Biología, UNAM
Apartado Postal 70-233, Coyoacán 04510, México D.F. México

Abstract—Macromycetes associated with four different vegetation types in the Pinacate and Great Altar Desert biosphere reserve were studied over a one-year period. Twenty-seven taxa representing the *Phallales* and *Agaricales* were determined. Families represented include the *Geastraceae* (4), *Agaricaceae* (5), *Phelloriniaceae* (1), *Lycoperdaceae* (4), *Schizophyllaceae* (1), and *Tulostomataceae* (12). Of these, *Montagnea arenaria* and *Podaxis pistillaris*, which produced the highest number of collections, were the most widely distributed; species belonging to the *Geastraceae* and *Lycoperdaceae* showed a more restricted distribution. The most species-rich genus was *Tulostoma* with 12 taxa. *Geastrum berkeleyi, G. schmidelii* and *Tulostoma mohavei* are new records for México. Chorology and phenology of all species are outlined, and SEM micrographs of basidiospores representing six species are provided.

Key words—Gasteromycetes, taxonomy, chorology, phenology, SEM

Introduction

Mexico ranks among the first three places in the world as far as biological wealth is concerned, with over 12% of the world's biota. In general, its topography and geographical location on the boundaries of confluence of the neo-arctic and neo-tropical zones explain the large variety of ecosystems and resultant biological diversity. The Pinacate and Great Altar Desert biosphere reserve, which is located 31°30'-32°30' N and 113°00'-114°30' W, comprises an area of 714,556.5 ha. The core area includes two discontinuous regions: the Sierra del Rosario in the reserve's northwestern edge (with 41,392.5 ha) and the Sierra El Pinacate in the reserve center and eastern portion (with 228,112.75 ha). The buffer zone comprises 445,051.25 ha.

Over 50% of Sonora's area, 185,431 km^2, corresponds to the Sonoran Desert, from which the remainder covers a portion of Baja California Sur in Mexico and Arizona and

California in the United States. This region is considered a tropical-subtropical desert with bi-modal rain patterns. Its climate, physiography, edaphology, and hydrography favor the establishment of a large diversity of species (Shreve & Wiggins 1975). The Sonoran Desert has four subdivisions, each of which has very particular features: the central gulf coast, the Sonoran plains, the Arizona high plateau, and the lower Colorado river valley.

The biological diversity thus far catalogued for the biosphere reserve is represented by the following species: 41 mammals, 184 birds, 43 reptiles, 4 amphibians, 2 native fresh water fish, 560 vascular plants, and 11 fungi, including six Myxomycetes—*Didymium dubium* Rostaf., *Badhamia melanospora* Speg., *Fuligo intermedia* T. Macbr., *Physarum notabile* T. Macbr., *P. robustum* (Lister) Nann.-Bremek. and *P. straminipes* Lister (Moreno et al. 2004)—and five Gasteromycetes—*Battarreoides diguetii* (Pérez-Silva et al. 1994), *Dictyocephalos attenuatus* (Peck) Long & Plunkett (Esqueda et al. 1998), *Tulostoma cretaceum*, *T. fimbriatum*, and *T. leiosporum* (Esqueda et al. 2004).

Our study expanded what is known about macromycetes diversity in the Pinacate and Great Altar Desert Biosphere Reserve. We report below on the 27 species recorded during our survey, of which 23 are new to the reserve and three are new to the Mexican mycobiota.

Materials and methods

The survey was conducted at Pinacate and Great Altar Desert biosphere reserve. Four types of vegetation were sampled seasonally from fall 2003 to summer 2004: microphyllous desert scrub, sandy desert vegetation, mezquital, and sarcocaule scrub. The 10 sites studied were geo-referenced with a Magellan GPS ProMark X (Magellan System Corp., San Dimas, CA) (Table 1).

The sites have a dry type climate of the very dry, semi-warm sub-type, which is bi-modal: November to April and May to October with temperatures of 6 to 21ºC and 18 to 36°C, and rainfalls from 0 to 100 mm and 50 to 100 mm, respectively. The areas are located within the Sonoran plain, Altar Desert sub-province, Sonoran mountain range (sierra) and plain. Topographically, the sites correspond to sierra (Los Tanques), sandy zones (Ejidos of Punta Peñasco, Los Norteños, Sierra Blanca and Cerro Lava), sierras and small hills (Papalote), and badlands (Ejido of San Juanico; Celaya, El Colorado and El Elegante Craters).

Taxonomic classification follows Kirk et al. (2001). Specimens were collected and conserved following conventional mycological techniques. Spores of most of the species were observed under a Jeol, JSM-5200 scanning electron microscope (SEM). Samples were studied after a treatment in a Polaron E-2000 equipment for 60 seconds at 1.2 Kv and 20 mA in an argon atmosphere until a 500 Å gold cover was obtained. Specimens are kept in the macromycetes collection of the Centro de Estudios Superiores del Estado de Sonora (CESUES) with some duplicates at the National Herbarium (MEXU).

This work was presented at the "Fungal Biodiversity Symposium", realized on March 4th, 2005 to commemorate the 60th anniversary as researcher of the Emeritus Professor Dr. Teófilo Herrera at the Institute of Biology, UNAM.

Table 1. Sampling localities in Pinacate and Great Altar Desert biosphere reserve

Localities	N	W	Altitude	Vegetation
Municipality of Sonoyta				
I. Sierra Los Tanques	31°46'12"	113°00'48"	371 m	MDS
II. Ejido Punta Peñasco	31°45'41"	113°15'57"	154 m	SDV
III. Papalote	31°55'44"	113°01'40"	307 m	M
IV. Crater Celaya	31°59'17"	113°27'20"	277 m	SS
V. Crater El Colorado	31°55'04"	113°18'44"	201 m	SS
Municipality of San Luis Rio Colorado				
VI. Cerro Lava	32°03'02"	113°33'33"	229 m	SDV
Municipality of Puerto Peñasco				
VII. Crater El Elegante	31°51'34"	113°22'55"	252 m	SS
VIII. San Juanico	31°50'01"	113°20'13"	198 m	SS
IX. Ejido Los Norteños	31°39'36"	113°19'37"	132 m	SDV
X. Sierra Blanca	31°31'32"	113°25'28"	60 m	SDV

Vegetation types: Microphyllous Desert Scrub (MDS); Sandy Desert Vegetation (SDV); Mezquital (M); Sarcocaule Scrub (SS).

Results and discussion

Phallales: Geastraceae—The family is characterized by gastrocarps with peridia that split into stellate rays, unbranched capillitia, and warty spores. Four taxa were determined within *Geastrum*, three of them found in the mezquital: *G. berkeleyi*, *G. kotlabae* and *G. schmidelii* (Table 2). *Geastrum berkeleyi,* which is close to *G. campestre* Morgan, *G. kotlabae,* and *G. pseudolimbatum* Hollós, was diagnosed based on the following features: a conical, plicate peristome, a coarse endoperidium, a short stalk and a non-hygroscopic exoperidium. Spores measure 5–6 μm diam.; SEM micrographs indicate that the epispore is composed of columnar processes with truncated apices that are about 0.7 μm high and occasionally confluent (Fig. 1). *G. berkeleyi* is a first record for Mexico.

Macroscopically, *Geastrum kotlabae* is similar to *G. campestre* and *G. pouzarii* V.J. Staněk, but differs by having a sessile spore sac in contrast to the stalked endoperidial sac in the other two species. In Mexico, it is known to grow in arid areas of Baja California, Tlaxcala and Sonora (Guzmán & Herrera 1969, Esqueda et al. 2003, Calonge et al. 2004). *G. kotlabae* was the only species collected throughout the year (Table 2). Within the Sonoran Desert, *G. minimum* has been found to grow in microphyllous desert scrub, tropical thorn forest, spiny scrubland and tropical deciduous forest (Pérez-Silva et al. 1999, Esqueda et al. 2003). In this specific study, it was collected from sarcocaule scrubland in spring.

The gastrocarp of *Geastrum schmidelii* resembles that of *G. pectinatum* Pers. but is smaller and has a short-stalked endoperidial sac covered by a thin powdery layer (this

Table 2. Distribution of macromycetes species in the Pinacate and Great Altar Desert biosphere reserve

SPECIES	LOCALITIES									
	I	II	III	IV	V	VI	VII	VIII	IX	X
Phallales, Geastraceae										
**Geastrum berkeleyi* Massee			4							
Geastrum kotlabae V.J. Staněk	2		1,2,3,4							
Geastrum minimum Schwein.							1			
**Geastrum schmidelii* Vittad.			3,4							
Agaricales, Agaricaceae										
Longula texensis (Berk. & M.A. Curtis) Zeller	2	3				3				
Chlorophyllum molybdites (G. Mey.) Massee			3							
Endoptychum arizonicum (Shear & Griffiths) Singer & A.H. Sm.		1							1	
Montagnea arenaria (DC.) Zeller	1,2,4	1,2,3,4	1,2,3,4	1,2,3,4	1,4	1,2,3	1	2	3,4	3,4
Podaxis pistillaris (L.) Fr.	2,3,4	1,2,4	1,2,3,4	4	1,2,4	1	1,2,4	3,4	1,2,3,4	1
Phelloriniaceae										
Phellorinia herculeana (Pall.) Kreisel emend. Demoulin	4									
Lycoperdaceae										
Abstoma stuckertii (Speg.) J.E. Wright & V.L. Suárez			2							
Calvatia pygmaea (R.E. Fr.) Kreisel et al.		1,2,4							2	

Season: 1 (Spring); 2 (Summer); 3 (Autumn); 4 (Winter). * New records for the Mexican mycobiota. See Table 1 for locality data.

Table 2 (Reserve macromycete distribution), concluded

SPECIES	LOCALITIES									
	I	II	III	IV	V	VI	VII	VIII	IX	X
Disciseda hyalothrix (Cooke & Massee) Hollós	2									
Disciseda verrucosa G. Cunn.			2,4							
Schizophyllaceae										
Schizophyllum commune Fr.			1,2,3,4							
Tulostomataceae										
Battarreoides diguetii (Pat. & Har.) R. Heim & T. Herrera					2				4	
Schizostoma laceratum Ehrenb.	1,2,4	1,2,4	2,3		4	1,4	1	1	1	
Tulostoma albicans V.S. White	2									
Tulostoma cretaceum Long		2				2			1	
Tulostoma fimbriatum Fr.			1,2,3						1	
Tulostoma involucratum Long							2			
Tulostoma leiosporum R.E. Fr.	4	2	1,4	2		1,4			1	
**Tulostoma mohavei* Lloyd	2									
Tulostoma nanum (Pat.) J.E. Wright		2		1			1,4		1	
Tulostoma obesum Cooke & Ellis		1	2	2,4	1	1,4	1,4	2,4	1	1,4
Tulostoma pygmaeum Lloyd	2									
Tulostoma xerophilum Long	2			1						

Season: 1 (Spring); 2 (Summer); 3 (Autumn); 4 (Winter). * New records for the Mexican mycobiota. See Table 1 for locality data.

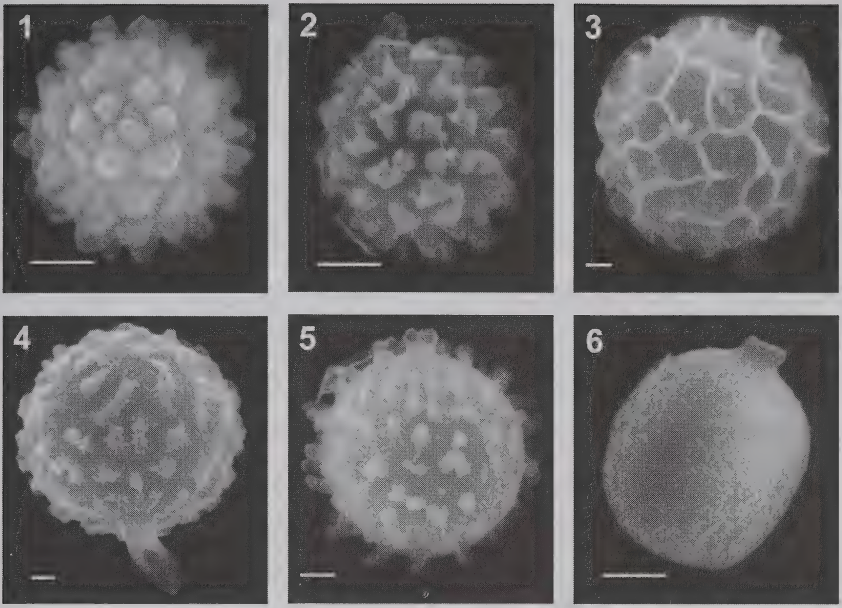
crystalline pruina is occasionally absent). Spores measure from 4.5 to 5.5 µm; SEM microphotos reveal an epispore formed of warts and columnar processes that are about 0.5 µm high and occasionally coalescent (Fig. 2). In Northwestern Europe, it grows in association with scrubland vegetation in open areas on well-drained sandy, slightly alkaline and calcareous soils (Sunhede 1989). These features are similar to those of the site, where it was collected in mesquite tree vegetation in the Sonoran reserve (Table 2). It is recorded for the first time in Mexico.

Agaricales: Agaricaceae—This family comprises 51 genera and 918 species. It is characterized by basidiomes with velar structures and spores of variable color but never rusty-brown or cinnamon-brown (Kirk et al. 2001). Five taxa were determined for *Agaricaceae*, with *Montagnea arenaria* and *Podaxis pistillaris* outstanding as they were present in all sites and found throughout the year at several sites. Both have smooth, thick-walled pigmented spores. These characteristics help to explain its wide distribution throughout the reserve under extreme conditions, as they are commonly found in areas with no vegetation at all. Apparently these fungi, together with species that belong to the *Tulostoma obesum* complex, are the best adapted in this ecological reserve. They have been registered for several xeric regions of Sonora (Aparicio-Navarro et al. 1994, Pérez-Silva et al. 1994, Esqueda et al. 1998).

Longula texensis, Chlorophyllum molybdites and *Endoptychum arizonicum* had a more restricted distribution. *Longula texensis,* which is similar to *Gyrophragmium dunalii* (Fr.) Zeller, is considered uncommon in Mexico (Ochoa 1993). Recently Geml et al. (2004), based on the sequence of internal transcription spacers (ITS) and largest partial sub-unit of ribosomal ADN, confirmed that these species are valid since they evolved from different *Agaricus* taxa. *Chlorophyllum molybdites* is widely distributed throughout Mexico and can cause gastrointestinal distress (Pérez-Silva 2004). It was restricted to areas of mezquital at the end of the rainy season during fall. Although *Endoptychum arizonicum* has been frequently collected in arid areas of Sonora (Esqueda et al. 1990, 1998), distribution in the reserve was restricted to two sites with sandy desert vegetation. This secotioid fungus with smooth and thick-walled spores is well-adapted to xeric environments.

*Agaricales: **Phelloriniaceae***—Diagnostic characters of this family include stipitate gastrocarps and basidia that persist in bundles in the mature gleba. Although *Phellorinia herculeana* is widely distributed throughout the world's arid regions, it is uncommon. In fact, this is the third record for Sonora: *P. herculeana* was previously cited for the microphyllous desert scrub (Aparicio-Navarro et al. 1994) and in spiny scrubland (Esqueda et al. 1998). Recordings for Mexico are scarce: San Luis Potosí, Sinaloa (Guzmán & Herrera 1969), Nuevo León and Tamaulipas (Urista et al. 1985). It was the only species observed growing in the microphyllous desert scrub, solitary during winter in the Pinacate (Table 2).

Agaricales: Lycoperdaceae—Family features include epigeous gasterocarps with apical openings, hyphae lacking clamp connections, and powdery gleba. The four representatives of the *Lycoperdaceae* that were determined had a restricted distribution and fruited during summer (Table 2). *Abstoma stuckertii* was found in association with



Figs. 1–6. SEM micrographs of basidiospores. Fig. 1. *Geastrum berkeleyi* (CESUES 5111). Fig. 2. *Geastrum schmidelii* (CESUES 5108). Fig. 3. *Abstoma stuckertii* (CESUES 5232). Fig. 4. *Disciseda hyalothrix* (CESUES 5208). Fig. 5. *Disciseda verrucosa* (CESUES 5226). Fig. 6. *Tulostoma mohavei* (CESUES 5209). Scale bar = 1 µm.

mezquital. The size and ornamentation of spores (Fig. 3) allow it to be easily identified. It is known to the United States, Argentina, and Australia (Suárez & Wright 1990).

Disciseda hyalothrix was only collected in microphyllous desert scrub. Gastrocarps of *Disciseda* taxa are similar and can be recognized by their distinctive spore ornamentation. *D. hyalothrix* is characterized by an epispore forming columnar processes that have flattened and confluent tips (Fig. 4). In Mexico, it has been recorded for xeric regions in Chihuahua (Laferriere & Gilbertson 1992), Sonora (Esqueda et al. 1995a) and Baja California (Ochoa et al. 2000).

The known distribution of *Disciseda verrucosa* on the American continent is restricted to Sonora, Mexico, where it is associated with microphyllous desert scrub (Aparicio-Navarro et al. 1994), spiny scrubland, and the low deciduous forest (Pérez-Silva et al. 2000). Spore ornamentation is very characteristic under SEM with an epispore composed of small warts and obtuse finger-like processes (Fig. 5). In the reserve, it was seen in mezquital during summer and winter.

Calvatia pygmaea is a xerophilous bovistoid species that belongs to *Calvatia* Section *Lanopila* (Fr.) Kreisel and is the only taxon in this group that has smooth spores under both LM and SEM. Worldwide, it is only known from Argentina and Bolivia (Fries 1909) and Baja California Sur, Mexico (Ochoa et al. 1998). It was restricted to sandy desert vegetation with gastrocarps collected in all seasons except autumn in the reserve (Table 2).

Agaricales: Tulostomaceae—Family characters include stipitate gasterocarps having long stalks with globose heads, peridia with apical pores, and pleurosporous basidia. *Battarreoides diguetii* has been previously registered for several sites in the Sonoran Desert (Pérez-Silva et al. 1994, Esqueda et al. 1995b). In the Pinacate, it was observed to grow in sarcocaule scrubland and sandy desert vegetation in summer and winter, respectively.

Schizostoma laceratum is only known to grow in Northwestern Mexico in Baja California (Moreno et al. 1995) and Sonora (Esqueda et al. 1995b). It showed a wide distribution within the reserve, growing in all types of vegetation, bearing gastrocarps mainly during spring (Table 2).

Different species of *Tulostoma* showed a restricted distribution: *T. albicans, T. mohavei* and *T. pygmaeum* were all found in microphyllous desert scrubland, and *T. involucratum* was found in sarcocaule scrubland (Table 2). In contrast, *T. leiosporum* and *T. obesum,* characterized by their subsmooth (rugose) and smooth spores under SEM, respectively, which have a thick and pigmented wall, had a wider distribution. *Tulostoma fimbriatum* was collected throughout the year, except for winter, while growing in mezquital. *Tulostoma mohavei* is similar to *T. obesum,* its difference being the ferruginous ochre colored gleba and the dark ferruginous to chocolate colored gleba, respectively. This is the first report for Mexico of *T. mohavei,* which previously had been cited in North America only from Arizona and California in the United States. The distribution of *Tulostoma pygmaeum* in Mexico was only known to Veracruz, Oaxaca (Wright 1987) and Baja California (Moreno et al. 1995). This is the first record for the Sonoran mycota.

Agaricales: Schizophyllaceae—This family comprises 5 genera and 43 taxa characterized by pleurotoid basidiomes with split-lamellate hymenophores (Kirk et al. 2001). *Schizophyllum commune* is widely distributed throughout Mexico from arid to template zones, occasionally in cold areas (Olivo & Herrera 1994). In the Pinacate, it was collected throughout the year only in mesquite tree vegetation (Table 2).

Acknowledgments

The authors thank SEMARNAT-CONACYT (Grant 2002-C01-0409) and DGAPA (Grant IN206901 (México). Our gratitude is to M.B. Mendoza-Garfias (UNAM) for her assistance with SEM. We are grateful to Prof. H. Kreisel and Prof. F.D. Calonge for the critical revision of the manuscript.

Literature Cited

Aparicio-Navarro A, Quijada A, Quintero T, Búrquez A. 1994. Nuevos Gasteromicetos para la micobiota de Sonora, México. Ecológica 3: 11-14.

Calonge FD, Guzmán G, Ramírez F. 2004. Observaciones sobre los Gasteromycetes de México depositados en los herbarios XAL y XALU. Boletín de la Sociedad Micológica de Madrid 28: 337-371.

Esqueda M, Quintero T, Pérez-Silva E, Aparicio A. 1990. Nuevos registros de Gasteromycetes de Sonora. México. Revista Mexicana de Micología 6: 91-104.

Esqueda M, Pérez-Silva E, Herrera T. 1995a. New records of Gasteromycetes for Mexico. Documents Mycologiques 98-100: 151-160.

Esqueda M, Pérez-Silva E, Villegas RE, Araujo V. 1995b. Macromicetos de zonas urbanas, II: Hermosillo, Sonora, México. Revista Mexicana de Micología 11: 123-132.

Esqueda M, Pérez-Silva E, Herrera T, Moreno G. 1998. Adiciones al conocimiento de los Gasteromicetos de Sonora, México. Revista Mexicana de Micología 14: 41-52.

Esqueda M, Herrera T, Pérez-Silva E, Sánchez A. 2003. Distribution of *Geastrum* species from some priority regions for conservation of biodiversity of Sonora, Mexico. Mycotaxon 87: 445-456.

Esqueda M, Moreno G, Pérez-Silva E, Sánchez A, Altés A. 2004. The genus *Tulostoma* in Sonora, Mexico. Mycotaxon 90: 409-422.

Fries RE. 1909. Über einige Gasteromyceten aus Bolivia and Argentinien. Arkiv for Botanik 8: 1-34.

Geml J, Geiser DM, Royse DJ. 2004. Molecular evolution of *Agaricus* species based on ITS and LSU rDNA sequences. Mycological Progress 3: 157-176.

Guzmán G, Herrera T. 1969. Macromicetos de las zonas áridas de México, II. Gasteromicetos. Anales del Instituto de Biología de la Universidad Nacional Autónoma de México, Serie Botánica 40: 1-92.

Kirk PM, Cannon PF, David JC, Stalpers JA. 2001. Ainsworth and Bisby's dictionary of the fungi. 9th ed. CAB International, Wallingford-Oxon.

Laferriere JE, Gilbertson RL. 1992. Fungi of Nabogame, Chihuahua, Mexico. Mycotaxon 44: 73-87.

Moreno G, Altés A, Ochoa C, Wright JE. 1995. Contribution to the study of the Tulostomataceae in Baja California, Mexico. I. Mycologia 87: 96-120.

Moreno G, Illana C, Esqueda M, Castillo A, Pérez-Silva E. 2004. Notes on Myxomycetes from Mexico II. Boletín de la Sociedad Micológica de Madrid 28: 55-63.

Ochoa C. 1993. Contribución al estudio taxonómico, ecológico y corológico de la clase Gasteromycetes *sensu lato* en Baja California, México. Doctoral Thesis. Universidad de Alcalá, Alcalá de Henares.

Ochoa C, Moreno G, Altés A, Kreisel H. 1998. *Calvatia pygmaea* (Gasteromycetes) in the deserts of Baja California Sur (Mexico). Cryptogamie Mycologie 19: 131-137.

Ochoa C, Moreno G, Altés A, Aguilar-Rodríguez JL. 2000. Gasteromycetes de Sierra Juárez, Baja California, México. I. Boletín de la Sociedad Micológica de Madrid 25: 157-166.

Olivo F, Herrera T. 1994. Las especies de *Schizophyllum* en México, su distribución ecológica e importancia etnomicológica. Revista Mexicana de Micología 10: 21-32.

Pérez-Silva, E. 2004. Intoxicación por hongos. 305-329, in OB Martínez (ed.), Intoxicaciones. México, McGraw-Hill-Interamericana.

Pérez-Silva E, Esqueda M, Herrera T. 1994. Contribución al conocimiento de los Gasteromicetos de Sonora. Revista Mexicana de Micología 10: 77-101.

Pérez-Silva E, Herrera T, Esqueda M. 1999. Species of Geastrum (Basidiomycotina: Geastraceae) from Mexico. Revista Mexicana de Micología 15: 89-104.

Pérez-Silva E, Esqueda M, Herrera T, Moreno G, Altés A. 2000. *Disciseda verrucosa* (Gasteromycetes) in Mexico. Mycotaxon 76: 337--341.

Shreve F, Wiggins IL. 1975. Vegetation and flora of the Sonoran Desert. Stanford University Press, Stanford.

Suárez V, Wright JE. 1990. South American Gasteromycetes IV. The genus *Abstoma*. International Journal of Cryptogamic Botany 1: 372-383.

Sunhede S. 1989. *Geastraceae* (Basidiomycotina). Morphology, ecology, and systematics with special emphasis on the North European species. Synopsis Fungorum 1. Fungiflora. Grønlands Grafiske A/S, Oslo.

Urista E, García J, Castillo J. 1985. Algunas especies de Gasteromicetos del Norte de México. Revista Mexicana de Micología 1: 471-523.

Wright JE. 1987. The genus *Tulostoma* (Gasteromycetes). A world monograph. J. Cramer, Berlin-Stuttgart.

MYCOTAXON

Volume 95, pp. 91–96 January–March 2006

A new species and a new record of *Lycoperdaceae* from India

Dipika Bisht[1], J.R. Sharma[1], Hanns Kreisel[2] & Kanad Das[3*]

[1]*Botanical Survey of India*
192, Kaulagarh Road, Dehradun 248195, India
[2]*Zur Schwedenschanze 4, D-17498 Potthagen, Germany*
**daskanad@yahoo.co.in*
[3]*Mycology Group, Agharkar Research Institute*
G.G. Agarkar Road, Pune 411004, India

Abstract—A new species, *Lycoperdon ovalicaudatum* and a new record, *Calvatia longicauda*, are described and illustrated in detail from Western Himalaya, India.

Key words—macrofungi, Gasteromycetes, *Lycoperdales*, taxonomy

Introduction

During the macrofungal investigation in Western Himalaya, authors collected some interesting specimens belonging to *Lycoperdon* Tourn.:Pers. and *Calvatia* Fr. Macroscopic and microscopic studies followed by literature survey revealed them as an undescribed species proposed herein as *Lycoperdon ovalicaudatum*, and *Calvatia longicauda* reported for the first time from India. The genus *Lycoperdon* is characterized by having basidiomata with more or less distinct pseudostipe, opening by apical pore and branched, rarely septate, capillitial hyphae. Out of ca 50 species known from the world over (Kirk et al. 2001), only a few have pedicellate spores. The genus *Calvatia* characterized by irregular dehiscence of peridium and regularly septate capillitium, comprises ca 36 species world wide (Kreisel 1994). Preliminary surveys have revealed rich representation of these genera in the Himalayan forests (Ahmad 1941, Gupta et al. 1974, Khare 1976, Thind & Thind 1982).

Materials and Methods

The present communication is based on the surveys undertaken to different localities of Uttaranchal during June–October in 2004 and 2005. Morphological characters were noted from the fresh specimens in the field. Microscopic anatomical details were studied from the dried material mounted in 5% KOH, lactophenol–cotton blue and lactic acid. Line drawings were prepared with the aid of camera lucida attachment at original magnification of 1500x for basidiospores, 500x for peridial structures and 500x or 1000x for capillitial hyphae. A total of 25 basidiospores were measured from the holotype. Colour terminology follows Kelly & Judd (1955). Herbarium name used follow Holmgren et al. (1990). Kr. refers to the personal herbarium of H. Kreisel.

Description of the species

Lycoperdon ovalicaudatum D. Bisht, J.R. Sharma & Kreisel, **sp. nov.**

Fig. 1

Etymology: The name refers to the ovate, pedicellate spores of this taxon.

Basidiomata pyriformia-turbinata, 18–22 mm lata, 20–24 mm alta. Pseudostipes praesens. Exoperidium griseofuscum, spinosum, spinae densae in parte superiori, sparsae in parte inferiori. Endoperidium pallide flavum, brunneum. Gleba griseofusca, pulverulenta. Pseudocolumella indistincta. Subgleba loculata. Basidiosporae ovales, 5.4–5.7 x 3.7–3.9µm, glabrae, guttulatae, pedicelli hyalini, recti, ad 22µm longi. Capillitium 4-6µm latum, fuscogriseum, crassitunicatum, non septatum, glabrum, ramosum, parietes non perforatae. Paracapillitium 3–4µm latum, hyalinum, septatum, tenuitunicatum. Exoperidium 500µm crassum. Endoperidium 60µm crassum, hyphis 2µm crassis, non septatis. ***Holotypus:*** *INDIA, Uttaranchal, Nainital, Sheetla, August 23 , 2004, leg. D. Bisht, DB514 (**HOLOTYPUS**, BSD; ISOTYPUS, Kr.).*

Basidiomata scattered or gregarious, pyriform to turbinate, 18–22 mm broad, 20–24 mm high. Rhizomorphs poorly branched. Pseudostipe present. Exoperidium dark greyish brown, spinose, spines up to 0.5 mm, dense, prominent on the upper part, scattered, blunt on pseudostipe. Endoperidium light yellowish brown, rough, opens by apical aperture. Gleba greyish brown, pulverulent at maturity. Pseudocolumella indistinct. Subgleba chambered, one- quarter of basidiomata in length, tapering towards base.

Basidiospores ovate, 5.4–5.7 x 3.7–3.9 µm, Q=1.5, smooth , guttulate, pedicellate, pedicels hyaline, long , curved, up to 22 µm in length. Capillitium greyish brown, 4–6 µm wide, smooth, aseptate, branched, thick-walled, wall more than 1µm thick, not pitted. Paracapillitium hyaline, 3–4 µm wide, smooth, septate, unbranched, thin-walled. Exoperidium pseudoparenchymatous, up to 500 µm thick, cells subglobose–oval, 20–40 x 14–32 µm in diameter, thin-walled. Endoperidium hyphal, up to 60 µm thick, hyphae narrow, up to 2 µm wide, aseptate, unbranched, thin-walled.

COLLECTIONS EXAMINED––**INDIA, Uttaranchal,** NAINITAL, Sheetla, on soil, August 23, 2004, leg. D. Bisht, DB514 (BSD).

Comments—*Lycoperdon ovalicaudatum* can be distinguished by comparatively smaller basidiomata, prominent spiny warts on the upper part, chambered subgleba and ovate, smooth, pedicellate spores. The shape and surface of basidiospores are unique which make this species distinct. The present taxon is close to *L. mundkurii* S. Ahmad but the latter has comparatively larger basidiomata with spines on exoperidium forming a dark central patch at the apical part. Further, *L. mundkurii* has verruculose spores with longer (30 µm) pedicels and much branched and wider (up to 10 µm wide) capillitial hyphae. *L. asperum* (Lév.) Speg. which also appears closer to the present taxon morphologically, has globose-subglobose spores and rudimentary subgleba (Bottomley 1948, Dissing & Lange 1962, Kreisel & Hausknecht 2002).

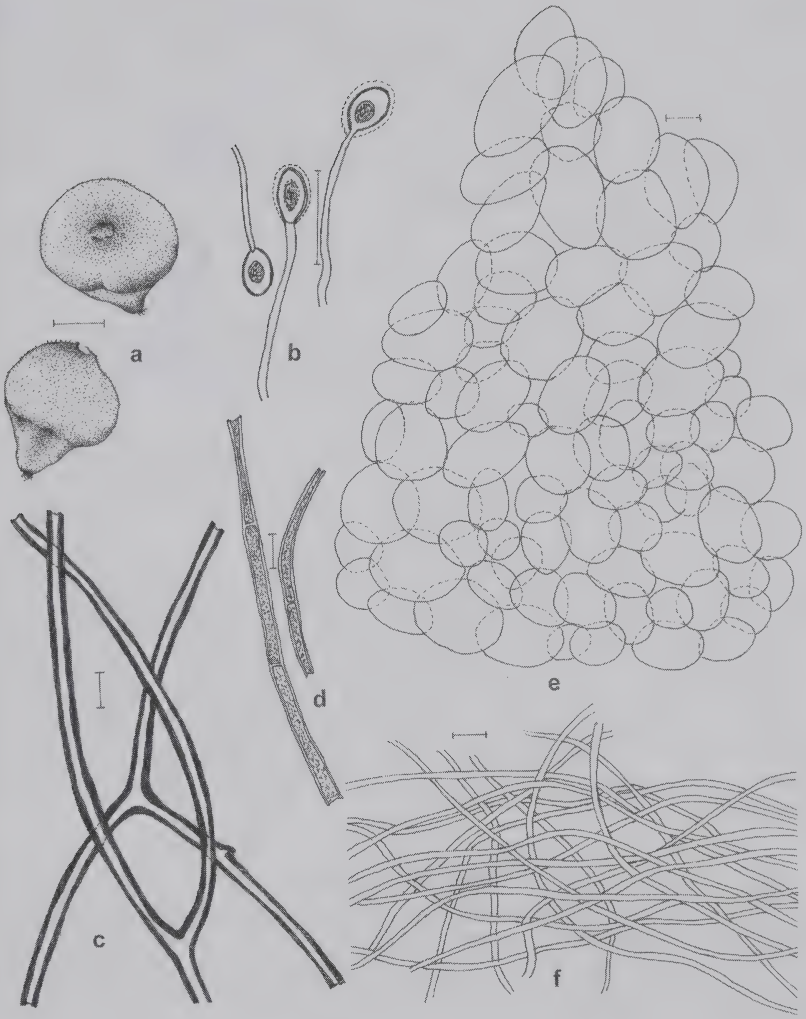


Fig. 1. *Lycoperdon ovalicaudatum* (from holotype): a. Basidiomata b. Basidiospores c. Capillitium d. Paracapillitium e. Exoperidium f. Endoperidium. Bars: a = 10 mm; b-f = 10 μm.

Calvatia longicauda (Henn.) Lloyd, Mycol. Writ. 2 (Letter 21): 3. 1908

=*Lycoperdon longicaudum* Henn., Botan. Jahrb. Syst. 23: 556. 1897

Fig. 2

Basidiomata scattered on leaf litter, turbinate-agaricoid, 4–8 cm broad, up to 7 cm high, distinctly lobed, irregular, flattened head with a long pseudostipe, fragile-brittle after drying. Rhizomorphs whitish, fine, profusely branched. Pseudostipe curved, up to 5 cm in length, tapering towards base. Exoperidium dark brown, verrucose with prominent verrucae on upper portion, shed off at maturity. Endoperidium light orange yellow, rough, peeling off in irregular flakes at maturity. Gleba medium yellow brown at centre, dark yellow brown beneath peridium, very persistent. Pseudocolumella indistinct. Subgleba dark yellowish brown, chambered.

Basidiospores globose-subglobose, 3.3–4.0 x 3.3–3.9 µm, verruculose, guttulate, apedicellate. Capillitium olivaceous yellow, up to 4 µm wide, lumen hyaline, septate, dichotomously branched, tapering towards ends, pitted, wall up to 1µm thick. Paracapillitium absent. Exoperidium pseudoparenchymatous, up to 625 µm thick, cells 10–25 x 7–15 µm, cylindrical, arranged in irregular chains on upper part, gradually subglobose, compact on lower part. Endoperidium hyphal, up to 125 µm thick, hyphae 2–4.5 µm wide, septate, branched, thin-walled.

COLLECTIONS EXAMINED—**INDIA, Uttaranchal,** DEHRADUN, Forest Research Institute campus (700 m), on soil, June 26, 2004, leg. D. Bisht & K. Das, DB1005 (BSD); ibid. Botanical Survey of India campus (700 m), on soil, October 8, 2005, leg. D. Bisht, DB605 (BSD).

Comments—The agaricoid basidiomata, ruptured peridium after drying and persistent gleba are the main determining features of this species. *Calvatia gardneri* (Berk.) Lloyd can be confused with this species in the field, but the earlier one lacks the persistent gleba and agaricoid basidiomata (Dring & Rayner 1967). *C. craniiformis* (Schwein.) Fr. resembles the present species in the manner of rupturing the peridium and in size and shape of basidiospores but differs in having wider (up to 7.7 µm) capillitial hyphae (Zeller & Smith 1964). Unlike *C. longicauda*, *C. candida* (Rostk.) Hollós has pulverulent gleba and pseudoparenchymatous exoperidium with cells not arranged in chains (Thind & Thind 1982).

Acknowledgements

The authors are thankful to the Director, Botanical Survey of India, Kolkata and Joint Director, Northern Circle, Dehradun for providing facilities during the present study. Prof. F.D. Calonge (Real Jardin Botánico, Madrid, Spain) and Prof. Gabriel Moreno (University of Alcalá de Henares, Spain) are duly acknowledged for critically reviewing the manuscript. Financial assistance to one of the authors (DB) is provided by Botanical Survey of India.

Literature Cited

Ahmad S. 1941. Higher Fungi of the Panjab Plains III. The Gasteromycetes. Jour. Ind. Bot. Soc. 20: 135–143.

Bottomley AM.1948. Gasteromycetes of South Africa. Bothalia 4: 473–810.

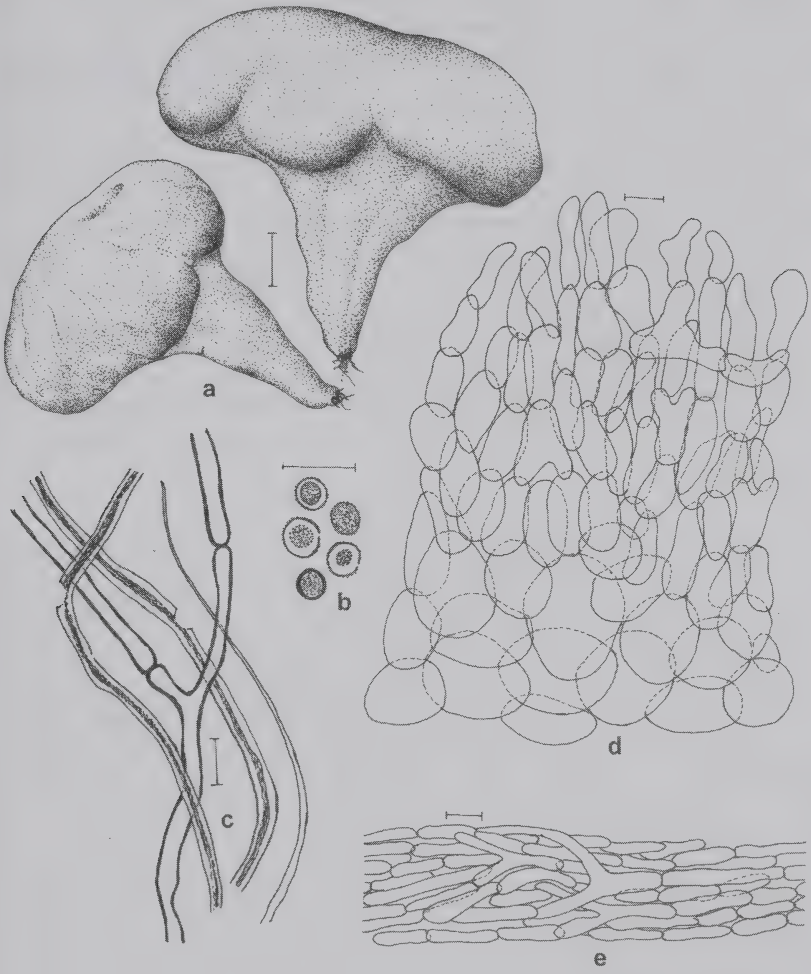


Fig. 2. *Calvatia longicauda*: a. Basidiomata b. Basidiospores c. Capillitium d. Exoperidium e. Endoperidium. Bars: a = 10 mm; b-e = 10 µm.

Dissing H, Lange M. 1962. Gasteromycetes of Congo. Bull. Jard. Bot. l'Etat 32(4): 325–416.

Dring DM, Rayner RW. 1967. Some Gasteromycetes from Eastern Africa. J. E. Afr. Nat. Hist. Soc. 26 no 2(114): 5–46.

Gupta KK, Agarwal RK, Kumar S, Seth PK. 1974. Gasteromycetes of Himachal Pradesh. Ind. Phytopath. 27(1): 45–48.

Holmgren PK, Holmgren NH, Barnett LC. 1990. Index Herbariorum. Part 1: Herbaria of the world, 86th ed. Brox: New York Botanical Garden.

Kelly KL, Judd DB. 1955. The ISCC-NBS Method of Designating Colors and a Dictionary of Colour Names. ISCC-NBS Color-Name Charts Illustrated with Centroid Colors. National Bureau of Standards Circular 553. U.S. Government Printing Office, Washington, DC.

Khare KB. 1976. Some Gasteromycetes from Uttar Pradesh. Ind. Phytopath. 29: 34–38.

Kirk PM, Canon PF, David IC, Stalpers JA. 2001. Ainsworth & Bisby's Dictionary of the Fungi. 9th edition, CAB International, Wallinford, 655pp.

Kreisel H. 1994. Studies in the *Calvatia* complex (Basidiomycetes) 2. Feddes Repertorium 105(5–6): 369–376.

Kreisel H, Hausknecht A. 2002. The gasteral Basidiomycetes of Mascarenes and Seychelles. Ősterr. Z. Pilzk. 11: 191–211.

Thind KS, Thind IPS. 1982. The Gasteromycetes of the Himalayas II. Jour. Ind. Bot. Soc. 61: 19–32.

Zeller SM, Smith AH. 1964. The Genus *Calvatia* in North America. Lloydia 27(3): 148–186.

MYCOTAXON

Volume 95, pp. 97–112 January–March 2006

Notes on the type, synonyms, and other specimens of the balansioid fungus, *Nigrocornus scleroticus*

Malcolm J. Ryley*

malcolm.ryley@dpi.qld.gov.au
Department of Primary Industries and Fisheries
PO Box 102, Toowoomba Qld, 4350 Australia

Abstract—The type, and other specimens of the balansioid fungus, *Nigrocornus scleroticus,* its synonyms, and similar fungi studied during extensive research on the taxonomy and biology of the fungus, are described. Two hypocrealean fungi found parasitising the ascostromata of *N. scleroticus* are also discussed.

Key words—*Balansia sclerotica, Ephelis, Nectriella, Bionectria*

Introduction

Ryley (2003) erected the genus *Nigrocornus* Ryley & Langdon, with *Nigrocornus scleroticus* as the type and only species, to accommodate a balansioid fungus formerly known as *Balansia sclerotica.* Both the teleomorph and anamorph of this fungus differ significantly from all other members of the tribe *Balansieae.* The teleomorph consists of perithecia immersed in a black, corniform, papillate stroma, composed of hyphae and the protophyll of the axillary bud, at the nodes of tillers of its grass hosts. The dimensions of the ascostromata vary considerably from host to host. The cylindrical, unitunicate asci within the perithecia have a hemispherical cap perforated by a fine pore, and contain eight filiform, 7-septate ascospores. Upon discharge from the asci, the ascospores produce short germtubes from each cell, and break into two 3-septate part-spores. The anamorphic conidia develop holoblastically on short, indeterminate conidiophores which are on an effuse hyphal layer covering the abaxial surfaces of the upper leaves on tillers bearing the teleomorph. Both the morphology and ontogeny of the anamorphic conidia of *N. scleroticus* are identical to those described for *Ephelis* Fr., which is the anamorph of the balansioid genera, *Balansia* Speg., *Dothichloë* G.F. Atk., and *Myriogenospora* G.F. Atk., and one of the two anamorphs of *Atkinsonella* Diehl (White, 1997; Hodge, 2003). *Nigrocornus scleroticus*, whose *Ephelis* fructification and sexual state are spatially separated, differs from all other described balansioid fungi. The anamorph(s) and teleomorphs of these fungi develop in succession on the same stroma (White, 1997).

---

*Formerly of the Department of Botany, University of Queensland, St. Lucia Qld 4072, Australia

Ryley (2003) has described, in detail, the development and morphology of *N. scleroticus*, host symptomatology, the fungus' relationships with other balansioid genera, and has formally erected the new genus. In this paper I provide information on specimens of the synonyms and other collections of *N. scleroticus*. In addition, the features of two hyperparasitic hypocrealean fungi, found on some ascostromata of *N. scleroticus*, are described and their identity is discussed.

Examination of herbarium specimens

All herbarium specimens were examined in a similar manner. The dimensions, shape and other aspects of the morphology of papillate ascostromata were ascertained, and where possible a single ascostroma was removed from the host and soaked for 24 hours in lactophenol. Sections 1-2 mm thick were taken from the ascostroma, and perithecia teased from the stromatic hyphae. Data were gathered on the morphology of perithecia, asci and ascospores. The uppermost leaves of the specimens, where present, were examined for the presence of effuse conidial fructifications. When such a fructification was found, a portion was mounted in lactophenol-trypan blue for 2 hours before examination.

The type specimen of *N. scleroticus*

Epichloë sclerotica Pat., J. Bot. (Morot) 4: 65 (1890)

Synonyms: *Ophiodothis sclerotica* (Pat.) Henn., Ann. Naturhist. Mus. Wien 15: 2 (1900).
Balansia sclerotica (Pat.) Höhn., Sitzungsber. Kaiserl. Akad. Wiss., Math.-Naturwiss. Kl., Abt. 1, 120: 449 (1911).
Parepichloë sclerotica (Pat.) J.F. White & P.V. Reddy, Mycologia 90: 231 (1998).
Nigrocornus scleroticus (Pat.) Ryley, Clavicipitalean Fungi: Evolutionary Biology, Chemistry, Biocontrol, and Cultural Impacts. pp. 267-268 (2003).

SPECIMEN EXAMINED–VIETNAM (as TONKIN). On ?*Cymbopogon* sp.: FAC-BIN 11.XI.1887 Balansa *FH* (**NEOTYPE**). Fig 1A.

Nigrocornus scleroticus is based on *Epichloë sclerotica*, which was described by Patouillard (1890) from a specimen collected in Tonkin, now Vietnam. The holotype of *E. sclerotica* could not be obtained from any herbarium, so the specimen listed above was designated as the neotype (see Ryley, 2003 for details). A complete description of Patouillard's original specimen is given by Ryley (2003). My observations on the teleomorph were almost identical with those of Patouillard (1890) except in some minor details. While Patouillard (1890), Hennings (1900), and Höhnel (1911) did not describe an anamorph for this species, Ryley (2003) found it to be present on the adaxial surfaces of the upper leaves of the neotype, and although in poor condition, to agree well with observations of the anamorph on fresh material of the fungus.

The type specimens of the synonyms and possible synonyms of *N. scleroticus*

1. *Hypocrella axillaris* Cooke, Grevillea 20: 4 (1891)

Synonyms: *Hypocrea axillaris* (Cooke) Massee, J. Bot. 34: 152 (1896).
Balansia axillaris (Cooke) Petch, Ann. Roy. Bot. Gard. (Peradeniya) 6: 171 (1916).

SPECIMENS EXAMINED–AUSTRALIA. QUEENSLAND. On Gramineae: BRISBANE FM Bailey *K ex Herb. FM Bailey 897&898*. On *Eragrostis pubescens* (R.Br.) Steud. (as *Eragrostis stricta* Cke.): WALSH R T Barclay-Millar *VPRI437 ex. Qld Herb.* (as *Eragrostis stricta*), .III.1891 T Barclay-Millar *DAR69754 ex Herb. FM Bailey 897 & 898* **(here designated LECTOTYPE). NORTHERN TERRITORY.** On *Chrysopogon violascens* Trin. (not a valid name; no known synonym): 20.IV.1916 GF Hill *VPRI438.*

Hypocrella axillaris was described by Cooke (1892) on grasses from Brisbane, Queensland, Australia, which had been collected by F.M. Bailey. The following description was provided by Cooke (1892): "Stroma obturbinate or obclavate, seated in the upper axils (5 mm long, 2-3 broad), black, opaque, minutely granular with the ostiola; substance white. Perithecia very minute, immersed in the periphery. Asci cylindrical, 120 μm long. Sporidia filiform, at length multiseptate (about 100 μ long), hyaline. On grasses, Brisbane (F.M. Bailey 897, 898)". Massee (1896) transferred the fungus to *Hypocrea* and considered that it was identical with *Hypocrea* (*Hypocrella*) *bambusae* Berk. & Broome. Some years later, Petch (1916) provided evidence by which the species *axillaris* and *bambusae* could be separated, and transferred both to *Balansia*. Petch (1916) added the following information on the morphology of the fungus – the stroma was adnate to the leaf (of the lateral shoot), the perithecia were flask-shaped, 0.15 mm deep and 0.15 mm diameter, and the asci were about 180 μm x 5 μm.

My examination of the specimens labelled F.M. Bailey 897 and 898 from several herbaria (*K*, *DAR*) has shown that the characteristics of that fungus published by Cooke (1892) and Petch (1916) were accurate, although the asci were closer to Petch's (1916) dimensions. On all specimens, a white flaky deposit was present on the adaxial surfaces of the uppermost leaves, but no acicular conidia were found. Some differences were observed in the stromata from material in the two specimens from Kew. In specimen number 897 the protophyll of the axillary bud at each node is attached to the stroma, whereas in number 898 the protophyll is not adnate to the stroma.

There is some evidence that Cooke's material was collected from at least two grass species. Firstly, Cooke (1892) described *H. axillaris* on "grasses". Secondly, large spines up to 1 mm long are present around the margin of the leaf blade on the host material in specimen 898, ex *K*. The spines are absent from leaf blades of the material in specimen 897 from that herbarium. The material in both specimens consists of only vegetative portions of the host, so the identity of the grasses cannot be ascertained with any certainty. One of the specimens in *DAR69754* consists of two short sections of culm held on a piece of card by a paper strap with the annotation "897 & 898". On each culm there is a single ascostroma, and a thin, dried, effuse hyphal sheet on the abaxial surfaces of the upper leaves. The long spines found in the Kew specimen 898 are absent from the leaves on both sections of culm, and the protophyll is adnate with stromal tissue on both ascostromata.

One of the hosts of this fungus is probably *Eragrostis pubescens* (R.Br.) Steud. (syn. *Eragrostis stricta* F.M. Bailey), because (i) Bailey (1913) listed it as the host of *Hypocrea axillaris*, (ii) in *VPRI437*, *E. stricta* is listed as the host of *Hypocrea* (*Hypocrella*) *axillaris* on a mounting board displaying a short length of a single culm with several ascostromata and the annotation "898" (iii) the annotation "Hypocrea (Hypocrella) axillaris on a grass (Eragrostis stricta) Walsh R, T.B. Millar" is on a specimen packet containing short sections of stem each with a single ascostroma in *DAR69754* and (iv) *Eragrostis stricta* was described by Bailey (1891) from material collected at Walsh River, north-eastern Queensland. Palmer et al., (2005) listed *E. stricta* as a synonym of *E. pubescens*. The locality on *VPRI437* and *DAR69754* is also given as Walsh River.

Taking these facts into consideration, it is likely that *VPRI437, DAR69754,* and the two specimens originally from F.M. Bailey's herbarium (897, 898) and now deposited at Kew, were collected at the same time near Walsh River in north-eastern Queensland by Barclay-Millar and examined by Bailey in Brisbane. One of the grasses is *E. pubescens*, but the other is unknown. Because the type specimen(s) of *B. axillaris* contains ascostromata on two different species of grasses, one of them must be designated as the lectotype. Cooke listed the specimens F.M. Bailey 897 and 898 as the specimens he examined to typify the fungus, and neither Massee (1896) nor Petch (1916) designated a type specimen. Specimen *DAR69754* is here designated as the lectotype because it is most representative of Cooke's original description.

The fungus called *B. axillaris* is very similar to *N. scleroticus*. The ascospores are somewhat shorter than those of the type of *N. scleroticus*, but in material which may possibly have been collected while immature, the significance of disparities in dimensions is probably less than the mere figures might suggest.

2. ***Epichloë oplismeni*** Henn., Bot. Jahrb. Syst. 22: 76 (1895)

Synonyms: *Ophiodothis oplismeni* (Henn.) Henn., Ann. Naturhist. Mus. Wien 15: 2 (1900).
Parepichloë oplismeni (Henn.) J.F.White & P.V.Reddy, Mycologia 90: 231 (1998).

SPECIMEN EXAMINED–CAMEROON (as KAMEROUN). On *Oplismenus* sp.: VICTORIA 25.III.1894 Preuss *B ex Preuss No.1153* (HOLOTYPE).

In his description of *Epichloë oplismeni,* Hennings (1895a) noted that the stromata were curved, black, 3-4 mm long and 1 mm wide. The asci were 180-220 x 5-5.5 μm, and the ascospores were 90-110 x 0.3-0.5 μm, aseptate and hyaline. Examination of the type specimen revealed that the ascospores are 7-septate, and 180-220 x 3-5 μm. During preparation of the teleomorph for microscopic examination the ascospores easily fragmented into 3-septate part-ascospores, as do those of *N. scleroticus*. No conidia were found on the upper leaves of the specimen, but hyphal sheets, similar to those found on many specimens of *N. scleroticus,* were present. The lack of conidia on the type specimen of *O. oplismeni* may be due to the weathered state of the specimen at the time of collection. I consider that *O. oplismeni* is a synonym of *N. scleroticus.*

3. ***Epichloë schumanniana*** Henn., Pflanzenw. Ost-Afrikas, C: 32 (1895)

Synonyms: *Ophiodothis schumanniana* (Henn.) Henn., Ann. Naturhist. Mus. Wien 15: 2 (1900).
Balansiopsis schumanniana (Henn.) Höhn., Sitzungsber. Kaiserl. Akad. Wiss., Math.-Naturwiss. Kl., Abt. 1, 119: 61 (1910).

SPECIMENS EXAMINED–CONGO (as BELGIAN CONGO). On *Schizachyrium brevifolium* (Sw.) Nees: Holst *B ex. Holst n. 3110* (HOLOTYPE) [No host name or locality was given on the type specimen packet, but in his original description Hennings (1895a) called the host *Schizachyrium brevifolium*, and gave the locality as East Africa]. On *Andropogon* sp.: .1913 T Enlleb *B*. On *Andropogon* sp.: Warnorki *B*.

Hennings (1895b) described the fungus as having curved stromata, 3-5 x 1 mm, asci 150-210 x 2-4 μm with an obtuse to rotund apex, and filiform, multiguttulate ascospores, 120-

180 x 0.5-1.0 µm. Höhnel (1910) considered that *O. schumanniana* should be transferred to *Balansiopsis* Höhn., an opinion based on the similarity of the development of the ascostromata of *Epichloë schumanniana* with that of *Balansiopsis gaduae* (Rehm.) Höhn., the type species of *Balansiopsis*. An important taxonomic criterion of the fungi assigned to *Balansiopsis* was the lack of an anamorph. However, that genus is no longer valid because *Ephelis* anamorphs have been described for all the fungi placed in *Balansiopsis* (White, 1997). My examination of the type specimen showed that Hennings' (1895b) description of the ascostromata and asci was accurate. The ascospores are 7-septate and 120-180 µm long. Conidia, 14-21 µm long and identical in all other respects with those of the type specimen of *N. scleroticus*, were found on the upper leaves of this specimen. Both the ascal and conidial states found on the type specimen of *B. schumanniana* are very similar to the corresponding states on *N. scleroticus*, so it can be considered to be conspecific with the latter. White & Reddy (1998) considered that *Epichloë sclerotica* (sic) and *Epichloë schumanniana* were identical, based on ascostroma morphology and ITS1 sequences.

4. *Epichloë volkensii* Henn., Pflanzenw. Ost-Afrikas, C: 32 (1895)

Synonyms: *Ophiodothis volkensii* (Henn.) Sacc., Bull. Soc. Roy. Bot. Belg. 38: 161 (1899).
Balansia volkensii (Henn.) E.Castell. & Cif., Prod. Mycofl. Afr. Or. p. 20 (1937).
Parepichloë volkensii (Henn.) J.F.White & P.V.Reddy, Mycologia 90: 231 (1998).

SPECIMENS EXAMINED–AFRICA. On *Andropogon* sp.: KILO? G Volkens *B* (**HOLOTYPE**). **CENTRAL AFRICA?** On *Anthephora* sp.: DJUR IX.1867 G Schweisfurth? *B*. **CENTRAL AFRICAN REPUBLIC (as FRENCH CONGO).** On grasses: KOUTI REGION (NEAR NDÉLE) 15.XI.1891 J Dybowski *F6844 ex Patouillard Herb. 597* (as *Hyalodothis clavus*).

Hennings (1895b) reported that the host of *Epichloë volkensii* was *Andropogon exothecus* Hack. On the packet containing the type specimen, and in the description of *Ophiodothis volkensii* (Bresadola & Saccardo, 1899), the host is given as *Andropogon* sp. In his paper, Hennings provided the following description: "Stromatibus vaginas ramulorum ambientibus subcylindraceis, curvatis, corniformibus, semini cornuto similibus, duris, atris, rugulosis, minute papillatis, usque ad 1 cm longis, 2 mm crassis, apicibus plerumque elongatis: peritheciis subglobosis; ascis cylindraceis, 8-sporis; sporidiis filiformibus. auf *Andropogon exothecus* - Volk.n.988". Castellani & Ciferri (1937) transferred the fungus to *Balansia*, but provided no reasons for their action. Roger (1953) reported that the perithecia of *B. volkensii* measured 225-375 x 90-135 µm and that 'Les asques cylindriques, octospores, diffluent tòt et contiennent des spores filiformes très allongées (90-150 µm) et fortement guttulées'. He gave no indication of the specimen he examined, so the validity of his report is questionable.

My examination of the type material confirmed much of Hennings' (1895b) data. Transverse sections of a stroma revealed that it has a structure similar to that of ascostromata of *N. scleroticus*. Immersed in the stroma are locules 360-390 x 120-150 µm which were empty of asci. No asci or ascospores were found in the type material, a situation which was undoubtedly influenced by the weathered nature of the ascostromata. The type specimen of *B. volkensii* lacks the upper portions of the tillers where an anamorph might possibly have developed on the unfolding leaves. Due to the inability to determine the morphology of both the anamorph and teleomorph of *E. volkensii*, it is here considered to be a tentative synonym of *N. scleroticus*.

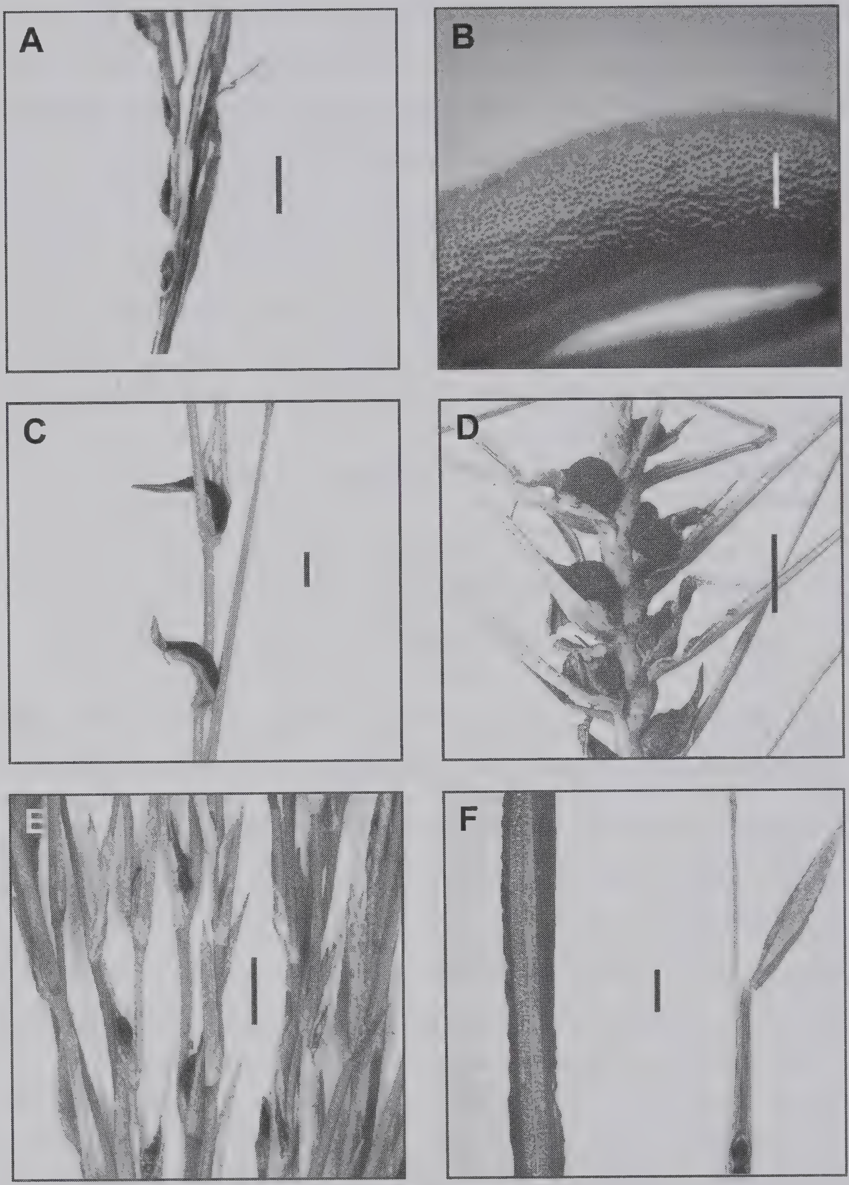


Figure 1. *Nigrocornus scleroticus.* **A** Portion of neotype with ascostromata (*FH*) (bar = 5 mm); **B** Papillate surface of ascostroma on *Sarga leiocladum* (*BRIP13402*) (bar = 1 mm); **C–E** Ascostromata on **C** *Sarga leiocladum* (*BRIP13402*) (bar = 5 mm), **D** *Triodia epactia* (*BRIP27802*) (bar = 10mm), and **E** *Entolasia stricta* (*BRIP13389*) (bar = 5 mm); **F** *Ephelis* anamorph on leaves of *Digitaria biformis* (*IMI123627*) (bar = 5 mm)

5. *Ophiodothis vorax* var. *paspali* Henn., Bot. Jahrb. Syst. 28: 274 (1901)

Probable synonym: *Balansia vorax* var. *paspali* (Henn.) Tesdoro (?)

> **SPECIMEN EXAMINED–JAPAN. SURUGA PROVINCE.** On *Paspalum scrobiculatum* L. (as *Paspalum filiculare* Nees on specimen packet): 18.X.1921 K Hara *L W ex Sydow fungi exotici exsiccati No. 520.*

Ophiodothis Sacc., type species *O. vorax* (Berk. & M.A. Curtis) Sacc., was based on *Dothidea vorax* Berk. & M.A.Curtis (Berkeley & Curtis, 1883). *Ophiodothis vorax* var. *paspali* was described by Hennings (1901) on *Paspalum filiculare* Nees from Japan with the following characteristics: Stromata in the axils of the culms, corniform, black, about 2 cm long, 2 mm wide; asci cylindrical, apex rotund, 8-spored, 90-120 x 5-6 μm; spores filiform, multiguttulate, 0.6-0.7 μm wide. Although the name *Balansia vorax* var. *paspali* (Henn.) Tesdoro is listed in Index Fungorum (www.indexfungorum.org/Names/Names.asp), no reference to a formal description could be found. A specimen labelled *Ophiodothis vorax* var. *paspali* was examined, but the type specimen was unobtainable from any of the many herbaria to which requests were directed. My examination of the available specimen revealed that the stromata are 12-20 x 1.5-2.0 mm, the asci 168-220 x 4-6 μm, and the ascospores filiform, 144-180 x 1.0-1.5 μm and 7-septate. However, no conidia typical of *N. scleroticus* were found on the upper leaves of the specimens. On ascal characters alone, this fungus closely resembles *N. scleroticus.*

6. *Ophiodothis arundinellae* Henn., Bot. Jahrb. Syst. 37: 162 (1906)

> **SPECIMEN EXAMINED–JAPAN.** On *Arundinella anomala* Steud.: UMAJIMURA .X.1904 Yoshinaga *S* (**HOLOTYPE**).

Hennings (1906) described *O. arundinellae* with the following characteristics: Stroma in the leaf axils, corniform, cylindrical, obtuse, black ca. 5 x 2 mm, verrucose-rugulose; perithecia densely crowded, ovoid to ellipsoidal; asci cylindrical, apex rounded-capitate, 8-spored, 120-150 x 4-6 μm; spores arranged in a parallel manner, filiform, multiguttulate or septate, hyaline, 1.5-2 μm wide. Hab. on culms of *Arundinella anomola* (sic; = *anamola*).

The type specimen of this fungus consists of a short section of tiller on which there are two black, curved ascostromata, 4-5 x 1.5-2.0 mm. The morphology of the ascus and of the ascospores could not be ascertained due to the weathered nature of the ascostromata. The specimen did not include any leaves from upper parts of tillers where an anamorph might possibly be found. The characteristics of the sexual state of *O. arundinellae* as described by Hennings (1906) are similar in most respects to those of *N. scleroticus.* Unfortunately, no information on the dimensions and septation of the ascospores or on the characteristics of a possible anamorph could be obtained, so this fungus can be considered to be a tentative synonym of *N. scleroticus.*

7. *Ophiodothis paspali* Henn., Bot. Jahrb. Syst. 37: 162 (1906) (nomen nudum)

> **SPECIMEN EXAMINED–JAPAN.** On *Paspalum thunbergii* Kunth ex. Steud.: TOKYO BOTANICAL GARDENS TOKYO 6.IX.1899 Kusano *S.*

Despite an extensive literature search, no formal description of this fungus was found. *O. paspali* appears here because it was mentioned in a short discussion at the end of the

formal description of *Ophiodothis arundinellae*: "Die Art steht der *O. paspali* P. Henn. nahe, ist aber durch die kürzeren, von den Blattscheiden umschlossenen Stromaten, die längeren Asken und die viel breiteren Sporen genugsam verschieden" (Hennings, 1906).

A specimen bearing this name was obtained from an herbarium (*S*). The ascostromata are black, curved, and 7-12 x 1.5-2.0 mm, but the morphology of the asci and ascospores could not be determined due to the weathered nature of the stromata. Conidia identical to those of *N. scleroticus* were found on the upper leaves of this specimen. The name *Ophiodothis paspali* Henn. can be considered a nomen nudum under Article 34 of the International Code of Botanical Nomenclature. The specimen may be an authentic collection of the fungus described by Hennings (1901) as *Ophiodothis vorax* var. *paspali*.

8. *Balansia cynodontis* Syd., Ann. mycol. 33: 234 (1935)

Synonym: *Parepichloë cynodontis* (Syd.) J.F. White & P.V. Reddy, Mycologia 90: 231 (1998).

> **SPECIMENS EXAMINED–SOUTH AFRICA.** On *Cynodon dactylon* (L.) Pers.: HARTEBEESTPORT DAM 25.V.1928 AM Bottomley *PREM23473* (**HOLOTYPE**); BUFFELSPOORT .III.1938 EM Doidge & Turner *BPI ex Sydow Fungi exotici exsiccati No. 996.*

Balansia cynodontis was described on a South African specimen of *Cynodon dactylon* by Sydow (1935). The following summary of the characteristics of this fungus has been derived from Sydow's data: ascostromata straight or curved, 5.0-10.0 x 0.5-0.75 mm, papillate part covering outer surface is of variable extent; perithecia oblong lageniform, 175-200 x 70-90 μm; asci 110-130 x 5.5-6.0 μm containing eight filiform spores about 1 μm in diameter.

My examination of the type material revealed that on most ascostromata there are several papillate portions separated from each other by non-papillate areas. A similar condition is found on some ascostromata of *N. scleroticus* on *Paspalidium criniforme* S.T. Blake collected in southern Queensland. The asci are 135-170 x 5-6 μm and have a hemisperical apical thickening, 3 x 3 μm, perforated by a fine pore. Only a few ascospores could be measured due to the weathered nature of most of the ascostromata, but the ascospores observed were 120-160 μm long and 7-septate. Hyaline, acicular, aseptate conidia 10-23 μm long were found on the adaxial surfaces of the uppermost leaves on infected tillers.

The teleomorph and anamorph of *B. cynodontis* are very similar to those of *N. scleroticus* in the following characteristics: ascostroma shape, perithecium morphology, ascus morphology, ascospore septation, and conidium morphology. The non-papillate areas on the ascostromata of the holotype may be due to differential maturation, because the entire surfaces of mature ascostromata of a recent collection of *N. scleroticus* from Zimbabwe (*BRIP39212*) were papillate. However the upper and lower limits of the ranges of both ascus length and ascospore length for *B. cynodontis* are less than the corresponding values for *N. scleroticus*. These differences may be due to the immature nature of the specimen or to genetic differences between the fungi. I consider that this taxon is conspecific with *N. scleroticus,* but examination of fresh material collected from *Cynodon dactylon* is warranted.

9. *Balansia trachypogonis* Doidge, Bothalia 4: 854 (1948)

> SPECIMENS EXAMINED–SOUTH AFRICA. On *Trachypogon plumosus* Nees: KAALFONTEIN 9.III.1916 IB Pole-Evans *PREM9543b* (HOLOTYPE), 2.II.1916 IB Pole-Evans *PREM9435* (PARATYPE), 9.III.1916 IB Pole-Evans *PREM28581* (PARATYPE), 27.III.1917 IB Pole-Evans *PREM10082* (PARATYPE); JOHANNESBURG DISTRICT, III.1931, AM Bottomley *PREM26607* (PARATYPE).

The characteristics of *Balansia trachypogonis* as described by Doidge (1948) are summarised as follows: ascostromata straight-curved, 1-3 cm x 2-3 mm, perithecia 300-480 x 70-120 μm; asci 150-200 x 5 μm; ascospores hyaline, filiform, multiseptate, almost equalling the ascus in length, 1.25-2.5 μm wide. My examination of the holotype and paratypes of *B. trachypogonis* confirmed the data given by Doidge (1948) as well as revealing previously undescribed characteristics. The apical thickening of asci of this fungus is not hemispherical as in *N. scleroticus,* but is in the form of a slight thickening of the ascus wall. As a consequence, the apical pore is much wider than on asci of *N. scleroticus.* The ascospores are 144-180 μm long, 1.0-1.5 μm wide, and 7-septate. On the upper, unfolding leaves, there are conidia, 13-23 μm long and 1.0-1.5 μm wide. Both the anamorph and teleomorph of *B. trachypogonis* are very similar to those of *N. scleroticus.* The ascostromata of *B. trachypogonis* are longer than those of the type specimen of *N. scleroticus,* that difference probably being influenced by the host. Although the ranges of ascospore length and conidium length of the former fungus lie within the corresponding ranges of *N. scleroticus,* the characteristics of the apical thickening of the asci closely resemble that described for *Myriogenospora* (Luttrell & Bacon, 1977; Bischoff & White, 2003). However, *B. trachypogonis* cannot be transferred to *Myriogenospora* because the stromata of the species in that genus develop in the rolled or folded leaves of grasses (Luttrell & Bacon, 1977; Bischoff & White, 2003). On this basis, *B. trachypogonis* is considered to be a tentative synonym of *N. scleroticus,* whose taxonomic status may change after examination of additional specimens.

10. *Balansia sclerotica* var. *deformans* Govindu & Thirum., Mycopathol. Mycol. Appl. 20: 300 (1963)

Govindu & Thirumalachar (1963) erected *Balansia sclerotica* var. *deformans* based solely on differences in symptomatology between *B. sclerotica*-infected hosts; on *Cymbopogon caesius* culms bearing ascostromata were shorter than healthy culms, while infected culms on other hosts were longer than normal. Such diversity in symptomatology between hosts infected by *N. scleroticus* is discussed in length by Ryley (2003). Although the type specimen of this taxon was not examined, the variety *deformans* is here considered to be synonymous with *N. scleroticus.*

11. *Balansia madagascariensis* Vienn.-Bourg., Ann. Inst. Natl. Agron., Sér. 3, 2: 25 (1964)

> SPECIMEN EXAMINED–MADAGASCAR. On *Paecilostachys* sp.: RANOMAFANA .I.1964 J Bosser *P* (HOLOTYPE).

The stromata on the type specimen are straight, black, 2.5-4.0 x 1.0 mm, surrounding the axillary buds on the culms. Asci (as measured by me) are 144-180 x 3-6 μm, but

Viennot-Bourgin (1964) described the asci as being 60-90 x 6-10 μm, perhaps because he measured immature asci. No intact ascospores were seen, all having broken while being teased from the asci. The fracturing of the ascospore to give part-ascospores is typical of *N. scleroticus,* as discussed in earlier sections. The part-ascospores, 60-72 x 1.0-1.5 μm, are pointed at one end and blunt at the other, and appear to be aseptate. The septum at the blunt end of the part-ascospores and the fracturing of the ascospores indicates that at least the thick central septum typical of ascospores of *N. scleroticus* was present. In addition, the material may not have been mature at the time of collection, and for that reason the thin septa of the part-ascospores were not in evidence. Hyphae similar to those found on many specimens of *N. scleroticus* were present on the adaxial surfaces of the uppermost unfolding leaves, but no conidia were found on these hyphae.

Although the teleomorph of *B. madagascariensis* is very similar to that of *N. scleroticus,* the lack of conidia on the upper leaves and the uncertainty of the ascospore septation make the reduction of that taxon to synonymy with *N. scleroticus* tenuous.

12. *Balansia triraphidis* (herbarium name)

> SPECIMENS EXAMINED–AUSTRALIA. NORTHERN TERRITORY. On *Triodia bitextura* Lazarides (as *Triraphis pungens* R.Br. on specimen packet): 34 MILE SIDING ON NT RAILWAY 15.1.1913 GF Hill *VPRI439.*

Brittlebank (1940) listed *Balansia* sp. as a pathogen of *Triraphis* in his "Australian Fungi Index", citing the source of the record as *VPRI* (Pascoe, pers. comm.). The specimen received from that herbarium had *Balansia triraphidis* on the packet, and despite an intensive literature search no formal description has been found. The specimen consists of one badly weathered, black ascostroma, 4 x 3 mm, surrounding an axillary bud. Although the morphology of the teleomorph could not be determined, handwritten notes in the specimen packet provide the following information: perithecia 370 x 140 μm, asci 190-210 x 6 μm, ascospores 8 per ascus, 96 x 1-1¼ μm and 120 x 1-1¼ μm, multiseptate. Until evidence to the contrary is found, this specimen is considered to be *N. scleroticus.*

Summary of the synonyms of *Nigrocornus scleroticus*

Nigrocornus scleroticus (Pat.) Ryley

≡ *Epichloë sclerotica* Pat.
≡ *Ophiodothis sclerotica* (Pat.) Henn.
≡ *Balansia sclerotica* (Pat.) Höhn.
≡ *Parepichloë sclerotica* (Pat.) J.F.White & P.V.Reddy
= *Hypocrella axillaris* Cooke
= *Epichloë oplismeni* Henn.
= *Epichloë schumanniana* Henn.
= *Ophiodothis paspali* Henn. (nomen nudum)
= *Balansia cynodontis* Syd.
= *Balansia sclerotica* var. *deformans* Govindu & Thirum.
= *Balansia triraphidis* (herbarium name)

?= *Epichloë volkensii* Henn.
?= *Ophiodothis vorax* var. *paspali* Henn.
?= *Ophiodothis arundinellae* Henn.
?= *Balansia trachypogonis* Doidge
?= *Balansia madagascariensis* Vienn.-Bourg.

Other specimens of *Nigrocornus scleroticus* examined

SPECIMENS EXAMINED–*ASIA AND PACIFIC REGION*: AUSTRALIA. **New South Wales.** On *Entolasia stricta* (R.Br.) Hughes: GLENBROOK 9.XII.1933 LR Fraser *DAR29874*; MOUNT TOMAH 21.V.1977 C Nuzum *DAR28740*, 9.VI.1977 J Walker *DAR30009*, 9.VI.1977 C Nuzum *DAR30011* (As *Hypocrella* sp. on all specimen packets). On *Entolasia marginata* (R.Br.) Hughes: GLENBROOK 09.XII.1933 L Fraser *DAR29874*. On *Oplismenus imbecillis* (R.Br.) Roem. & Schult.: MOUNT TOMAH 6.IV.1977 R Keogh *DAR28742*, 21.V.1977 C Nuzum *DAR28741* (As *Hypocrella* sp. on both specimen packets). On *Triodia* sp.: PILLIGA SCRUB 8.X.1918 JB Cleland *DAR256* (As *Epichloë typhina* on specimen packet). **Northern Territory**. On *Eriachne mucronata* R.Br.: BICKERTON ISLAND 17.VI.1948 RL Specht *BRIP2817*; CORKSCREW PASS 4.VII.1948 ST Blake *BRIP2811 DAR21400* (as *Balansia* sp.). On *Sarga plumosum* (R.Br.) R.E.Spangler [as *Sorghum plumosum* (R.Br.) P.Beauv.]: STUART HIGHWAY 30 KM N DALY WATERS, 17.III.2000 RG Shivas IT Riley C&K Vanky *BRIP26983*. On *Schizachyrium pachyarthron* C.Gardner: FLORENCE FALLS (LITCHFIELD) 13.III.2000 RG Shivas *BRIP27878*. On *Sorghum* sp.: KATHERINE 17.III.2000 RG Shivas *BRIP27702*. On *Triodia basedowii* Pritzel: FINKE R 02.IV.2002 P Latz *DAR60915*. On *Triodia burbidgeana* S.W.L.Jacobs: PORT BRADSHAW 18.XII.1998 RG Shivas *BRIP25528*. On *Triodia procera* R.Br.: GROOTE EYLANDT 7.V.1948 RL Specht *BRIP2818*. On *Triodia pungens* R.Br.: ULURU 26.V.1979 P Latz *DAR61057*. On *Triodia shinzii* (Henrard) Lazarides: NNW ALICE SPRINGS 26.III.2000 C&K Vánky *BRIP27466*. **Queensland.** On *Bothriochloa ewartiana* (Domin.) C.E.Hubb.: KIDSTON VIII.1954 SL Everist *BRIP2808 DAR 21409* (as *Balansia* sp.). On *Chrysopogon fallax* S.T.Blake: 10 KM S GLADSTONE 25.IV.2004 RG Shivas *BRIP39883*; KANGAROO HILLS (INGHAM) 27.V.1965 IC Tommerup *BRIP2809 DAR 23783* (as *Balansia* sp.); E MAREEBA 24.III.2005 TS Marney *BRIP46794*; 24 KM N MT MOLLOY 02.V.2005 TS Marney *BRIP45836*. On *Cymbopogon refractus* (R.Br.) A.Camus: (KENMORE) BRISBANE 15.III.1981 MJ Ryley *BRIP13388**. On *Eremochloa bimaculata* Hack.: GEEBUNG (BRISBANE) 05.IV.2003 RG Shivas *BRIP39619*. On *Entolasia stricta* (R.Br.) Hughes: BRISBANE VI.1968 R Creagh *BRIP2810*; CHAPEL HILL (BRISBANE) 3.IX.1980 MJ Ryley *BRIP13389**. On *Eragrostis pubescens* (R.Br.) Steud. (as *Eragrostis oxylepis* Torr.): WALSH R .III.1891 T Barclay-Millar *DAR69754* (see *Hypocrella axillaris* above). On *Panicum* sp.: BEATRICE R (PALMERSTON) 9.III.1941 LG Miles *BRIP2813 DAR 21408* (as *Balansia* sp.). On *Heteropogon contortus* (L.) P.Beauv. ex Roem. & Schult: 20 KM N GIN GIN, 25.IV.2003 MDE & RG Shivas *BRIP47180*. On *Paspalidium criniforme* S.T.Blake: HIRSTGLEN 8.III.1979 MJ Ryley *BRIP13390**, 26.VIII.1980 MJ Ryley *BRIP13391**, 9.IV.1981 MJ Ryley *BRIP 13392*, 11.IV.2003 MJ Ryley *BRIP39912*, .2003 MJ Ryley *BRIP45139*. On ? *Paspalidium distans* (Trin.) Hughes: JULATTEN 30.IV.1987 JL Alcorn *BRIP15816*. On *Paspalum scrobiculatum* L.: MT COTTON (BRISBANE) 6.I.1980 MJ Ryley *BRIP13393**, 4.II.1981 MJ Ryley *BRIP13394*. On *Paspalidium* sp.: MAREEBA WETLANDS (NEAR MAREEBA) 01.V.2004 MDE Shivas *BRIP44114*. On *Sarga leiocladum* (Hack.) R.E.Spangler [as *Sorghum leiocladum* (Hack.) C.E.Hubb.]: BLACKBUTT CK (BLACKBUTT) 1.IV.1979 MJ Ryley *BRIP13399*; COOYAR 1.IV.1979 MJ Ryley *BRIP 13400**, 2.III.1991 M Ryley *BRIP17463*; CROWS NEST 22.IX.1977 MJ Ryley *BRIP13396*; HIRSTGLEN 9.IX.1977 MJ Ryley *BRIP13395*, 19.XII.1977 RF Langdon *IMI 224366*, 10.XI.1978 MJ Ryley *BRIP13398*, 29.XI.1979 MJ Ryley *BRIP13401*, 28.III.1980 MJ Ryley *BRIP 13403***, 9.IV.1981, MJ Ryley *BRIP 13404**; KENMORE (BRISBANE) EX HIRSTGLEN 1.IX.1977 MJ Ryley *BRIP 15373;* J BJELKE-PETERSEN RESEARCH STATION (KINGAROY) 7.XII.2000 M Ryley *BRIP27613;* MAIDENWELL 13.XII.2000 RG Shivas MJ Ryley *BRIP27596;* MAPLETON 6.XI.1978 MJ Ryley *BRIP13397;* ST LUCIA (Brisbane) EX HIRSTGLEN 27.III.1980 MJ Ryley *BRIP13402**. On *Sarga plumosum* (R.Br.) R.E.Spangler [as *Sorghum plumosum* (R.Br.) P.Beauv.]: ATHERTON .IV.1960 G Keefer *BRIP2815 DAR 21411* (as *Balansia* sp.). On *Themeda triandra* Forssk. [as *Themeda australis* (R.Br.) Stapf]: BULLERINGA NEAR CHILLAGOE 24.V.2002 PR Trevorrow PR O'Keefe *BRIP29111;* MT NEBO 10.II.1981 MJ Ryley *BRIP13405**; MT TAMBORINE 21.IV.1962 DK Campbell *BRIP2816 DAR 21412* (as *Balansia* sp.). On *Triodia* sp.: 266 KM N CHARTERS TOWERS .V.2004 TS Marney *BRIP44584;* 40 KM W OF JERICHO 24.II2004 DR Beasley *BRIP43737;* TORRENS CK 26.III.1980 P Curran *BRIP13406.* On ? *Bothriochloa* sp.: 150 KM NW CHARTERS TOWERS .IV.1989 J&O Matthews *BRIP16640**. On unknown hosts: 241 KM N CHARTERS TOWERS 06.V.2004 TS Marney *BRIP44582;* MUSGRAVE 15.VII.1999 M Gunther *BRIP26753;* RIFLE CK (NEAR MT MOLLOY) 30.IV.1987 JL Alcorn *BRIP15784.* **WESTERN AUSTRALIA**. On *Sarga timorense* (Kunth.) R.E.Spangler [as *Sorghum stipoideum* (Ewart & White) Gardner & C.E.Hubb.]: POINT SPRINGS (NEAR KUNUNURRA) 8.X.1996 AA Mitchell *BRIP27800,* AA Mitchell *BRIP28853.* On *Triodia bynoei* (C.E.Hubb.) Lazarides: MITCHELL PLATEAU 10.II.1986 R Shivas *PERTH741884.* On *Triodia epactia* S.W.L.Jacobs: ONSLO(W) 18.V.1997 AA Mitchell *BRIP27802.* On *Triodia* sp.: BEAGLE BAY 1.III.2001 AA Mitchell *BRIP39323;* KARIJINI 11.VIII.2005 MJ Ryley *BRIP46838,* KARRATHA 09.VIII.2005 MJ Ryley *BRIP46839;* OOGALANOONGOO 3.VI.1996 AA Mitchell *BRIP27801.* **CHINA. YUNNAN.** On *Andropogon* sp.: .1919 AV Brooks *IMI182686**. On *Pogantherum crinitum* (Thunb.) Kunth [as *Pogantherum paniceum* (Lam.) Hack.]: TENGUYEH .X.1919 G Forrest *IMI4388 ex G. Forrest No. 18549.* **JAPAN.** On *Paspalum thunbergii* Kunth ex Steud.: SHIZOUKA 18.X.1921 K Hara *IMI4367 IMI22213 ex Sydow Fungi exotici exsiccata** (as *Ophiodothis vorax* var. *paspali* on specimen packet). **NEW GUINEA.** On *Themeda triandra* Forssk. [as *Themeda australis* (R.Br.) Stapf]: DARU ISLAND 5.VI.1957 F Kleckham *IMI74093.* **TONGA.** On *Cyrtocorrum oxyphyllum* (Hochst. ex Steud.) Stapf: WA Sykes *PDD47078**. ***INDIAN SUBCONTINENT.*** **INDIA.** On *Andropogon* sp.: DHERWAR .XI.1964 VS Seshadri *IMI116178**; NAGPUR 1.X.1922 RM Pearl *IARI21105.* On *Brachiaria distachya* (L.) Stapf: EX BANARAS HINDU UNIVERSITY .IX.1962 RA Singh *IMI123625**. On *Cymbopogon martinii* (Roxb.) J.F.Watson: EX URKRAM UNIVERSITY (UJJAIN) 27.II.1979 BS Sharma *IMI235875**. On *Dichanthium caricosum* (L.) A.Camus: EX BANARAS HINDU UNIVERSITY .X.1965 RA Singh *IMI123626**. On *Digitaria biformis* Willd.: EX BANARAS HINDU UNIVERSITY .IX.1963 RA Singh *IMI123627**. On *Ischaemum* sp.: MUNNAR KERALA DISTRICT 17.I.1965 T Raghunatti *IMI116179**. On *Setaria pumila* sub sp. *pallide-fusca* (Schum.) B.K.Simon [as *Setaria pallide-fusca* (Schum.) Stapf & C.E.Hubb.]: EX BANARAS HINDU UNIVERSITY .IX.1963 RA Singh *IMI123629.* On *Themeda* sp.: ORISSA 3.IX.1949 HF Mooney *IMI44594**. ***AFRICA*: GHANA (AS GOLD COAST COLONY).** On *Anadelphia* sp.: AYIKUMA 2.VI.1949 GJ Hughes *IMI46347**. **MALAWI (AS NYASALAND ON SOME).** On *Hyparrhenia dissoluta* (Nees) C.E.Hubb.: NENO 2.VII.1949 PJ Wuhl? *IMI38763;* ZOMBA 7.V.1949 PJ Wuhl? *IMI35550**. On *Hyparrhenia* sp.: LILONGWE 11.III.1964 DCM Corbett *IMI114417**; ZOMBA 19.III.1927 EJ Rutter? *IMI75064**. **NIGERIA.** On *Andropogon* sp.: OYO PROVINCE 6.VI.1954 RM Jarbron? *IMI56874**. **SIERRA LEONE.** On *Andropogon gabonensis* Stapf: NEWTON 6.VIII.1939 FC Deighton *IMI4385**. On *Andropogon tectorum* Schum. & Thonn.: NEWTON 6.VIII.1939 FC Deighton *IMI 4384**; HILL STATION 27.VII.1941 FC Deighton *IMI10965**; KEINADUGU DISTRICT .VIII.1963 H Mead *IMI112570**; MUSAIA 1.VII.1948 FC Deighton *IMI32562**; REGENT 11.VIII.1955 CT Pyle *IMI61714**; ROKUPR 25.VII.1939 FC Deighton *IMI4383**. On *Oplismenus hirtellus* (L.) P.Beauv.: KORTRIGHT 17.VIII.1958 FA Melville & T

Horker *IMI77026;* NJALA 18.VII.1927 FC Deighton *IMI4607* * (As *Epichloë oplismeni* on both specimen packets). **TANZANIA.** On ? *Hyparrhenia* sp.: PONGWE TANGA .IV.1970 DJ Allen *IMI158973**. **ZAMBIA (AS NORTHERN RHODESIA ON SOME).** On *Diheteropogon amplectens* (Nees) W.D.Clayt. (as *Andropogon amplectens* Nees): CHILANGA 4.III.1963 A Angus *IMI112416**; KAFUE GAME RESERVE 27.IV.1962 A Angus *IMI100139;* KASAMA 24.III.1960 A Angus *IMI119807.* On *Digitaria* sp.: MAZABUKA 2.IV.1962 A Angus *IMI100111.* **ZIMBABWE (AS RHODESIA ON *IMI 182949*).** On *Cynodon dactylon* (L.) Pers.: MATOPOS RESEARCH STATION .II.2002 DA Frederickson *BRIP39212**. On *Setaria pumila* sub sp. *pallide-fusca* (Schum.) B.K.Simon [as *Setaria pallide-fusca* (Schum.) Stapf & C.E.Hubb.]: QUE QUE 19.I.1974 A Rothwell *IMI182949.* **UNKNOWN.** On *Brachiaria distachya* (L.) Stapf: *IMI 4387.*

On these specimens the ascostromata are black, corniform, curved or straight, papillate, and variable in length and width (Figs. 1B-E). At one extreme are ascostromata on *Themeda triandra* (*IMI74093*) which measure 15-17 x 3-4 mm and at the other are those on *Oplismenus hirtellus* (*IMI4607*), being 2-3 x 1.0-1.5 mm. For most specimens the length:width ratio of ascostromata is >2:1, but on the specimens of *Triodia* species collected in Australia the ratio is very close to 1:1. The characteristics of the perithecia, asci and ascospores on ascostromata which could be examined are, in most cases, identical to those of the corresponding characteristics of the type specimen. For three specimens, *IMI4367, IMI22213* (both on *Paspalum thunbergii* from Japan), and *IMI114417* (on *Hyparrhenia* sp. from Malawi), the ranges of ascospore length (160-280 μm) are different to that of the type specimen (140-220 μm). A dried hyphal matrix was found covering the abaxial surfaces of the uppermost leaves on most specimens (Fig. 1F), and on some of these (marked * in the specimen list above) there were conidia identical in all respects to those found in the hyphal matrix on the type specimen.

Other specimens received as *Balansia sclerotica*

A number of specimens received as *Balansia sclerotica* from IMI were not conspecific with *N. scleroticus*. Specimens *IMI4449, IMI4478,* and *IMI4481* were prepared slides consisting of transverse sections of immature ascostromata with characteristics similar to *N. scleroticus.* The specimen packet of *IMI4478* indicates that the slide was made from specimen number 18549 of the collection of George Forrest; ascostromata of that specimen are deposited as *IMI4388* (see specimen list above). *IMI118290* and *IMI138634* were dried cultures from which conidia, 13-15 x 1.0-1.5 μm and with characteristics similar to those of the anamorph of *N. scleroticus,* were gathered. *IMI112156* consists of a conidiostroma of *Ephelis* sp. *IMI75075* is a smut fungus, while the other three (*IMI48964, IMI48969* and *IMI98181*), all collected on *Panicum congoense* Franch. from western Africa, have characteristics typical of *Parepichloë cinerea* (Berk. & Broome) J.F. White & P.V. Reddy (1998).

SPECIMENS EXAMINED–CHINA. YUNNAN PROVINCE. On *Pogantherum crinitum* (Thunb.) Kunth [as *Pogantherum paniceum* (Lam.) Hack.]: .1917-1919 *IMI4478 ex G. Forrest No. 18549.* **INDIA.** On *Cymbopogon martinii* (Roxb.) J.F. Watson: 20.IV.1966 MJ Thirumalachar *IMI118290.* On unidentified host: POONA MJ Thirumalachar *IMI138634.* **MALAYSIA.** On *Brachiaria mutica* (Forssk.) Stapf: SELANGOR .1897 Ridley *IMI4481* (As *Epichloë volkensii* on specimen packet). **NEW GUINEA.** On *Digitaria smutsii* Stent: AIYURA 19.II.1965 D Petty *IMI112156.* **REPUBLIC OF GUINEA.** On *Panicum congoense* Franch.: PELLEL KOURA

6.XI.1962 P Adames *IMI98181*. **SIERRA LEONE.** On *Panicum congoense* Franch.: GHAP? 12.X.1951, HD Jordan *IMI48964*, KASANKO 24.X.1951 HD Jordan *IMI48969*. **THAILAND (AS SIAM).** On *Cymbopogon nardus* (L.) Rendle: 3.VIII.1925 Garret *IMI4449*. **UGANDA.** On *Hyparrhenia cymbaria* Stapf: KAWANDA .VI.1944 CG Hansford *IMI75075*.

Hypocrealean fungi associated with *Nigrocornus* ascostromata

1. *Nectriella balansiae* R.H.Arnold, Mycologia 59: 248 (1967)

SPECIMENS EXAMINED–AUSTRALIA. QUEENSLAND. On ascostromata of *Nigrocornus scleroticus:* KENMORE (BRISBANE) 15.III.1981 MJ Ryley *BRIP13495* (On *Cymbopogon refractus*). **CONGO.** On ascostromata of *Nigrocornus scleroticus:* 15.XI.1891 J. Dybowski (as *Hyalodothis clavus*: HOLOTYPE *F 6844 ex Patouillard Herb. 597*) (grass host unknown).

Hyalodothis Pat. & Har. was described by Patouillard & Hariot (1893) with *H. clavus* Pat. & Har. as the type, and only, species. Diehl (1950) examined the type specimen of *H. clavus* and considered it to be "a species of *Balansia* identical with the African *Epichloë volkensii* P. Henn.". As a consequence he reduced *Hyalodothis* to synonymy with *Balansia.* Later, Arnold (1967) examined the type specimen of *H. clavus* and found small (100-165 x 60-100 µm), spherical-ovoid perithecia immersed in the ascostromata, where they were scattered between those of the *Balansia* species. She also rejected *Hyalodothis* and *H. clavus* on the grounds that the original descriptions included elements of *Balansia volkensii* and another fungus, and named the latter *Nectriella balansiae* R.H.Arnold, without describing an anamorph. Rossman et al., (1999) re-examined the type specimen and confirmed Arnold's description, with only minor modifications. During this study, perithecia which matched the description of *N. balansiae* were found in ascostromata on the type specimen of *H. clavus* and on an Australian specimen of *N. scleroticus* (*BRIP13495*), but no mature asci or ascospores were found in either.

Until evidence to the contrary is found, the Australian specimen is designated as *Nectriella balansiae*.

2. *Bionectria* sp.

SPECIMENS EXAMINED–AUSTRALIA. NEW SOUTH WALES. On ascostromata of *Nigrocornus scleroticus* (all as *Hypocrella axillaris*): MOUNT TOMAH 21.V.1977 C Nuzum *DAR28740* (On *Entolasia stricta*), 9.VI.1977 J Walker *DAR30009* (On *Entolasia stricta*), 9.VI.1977, C Nuzum *DAR 30011* (On *Entolasia stricta*), 6.IV.1977 R Keogh *DAR28742* (On *Oplismenus imbecillis*), 21.V.1977 C Nuzum *DAR28741* (On *Oplismenus imbecillis*).

Walker (from annotations, slides and cultures in specimen packets) found light yellow, raised, solitary or gregarious, dome-shaped perithecia of a *Nectria*-like species on some *Nigrocornus* ascostromata on the specimens listed above. On all of these specimens except *DAR30011* he also found the sporodochia of a fungus which he ascribed to *Myrothecium* Tode. My examination of the specimens showed that the conidia of the latter fungus are hyaline, thin-walled, aseptate, ellipsoidal, straight or slightly curved, with a distinctive hilum and 10-15 x 2.2-2.5 µm. The conidiophores are penicillate with two phialides arising from the supporting cell, each phialide being 3-4 µm wide at the widest point, narrowing to approx. 2 µm at the tip. On a 1-month-old culture of *DAR28741(b)* there are discrete, raised sporodochia with a dark green spore mass. The conidia are more variable than those on the host tissue, being cylindrical-ovate, 10-15 x 3-4 µm, but with a prominent protruding hilum. The asci in the *Nectria*-like perithecia are clavate, thin-walled, unitunicate, and lack any apparent apical thickening. There were 8 ascospores per ascus, the ascospores being hyaline, thin- and smooth-walled, 1-septate, fusiform, 10-15 x 2.5-3 µm, and with a slight constriction at the septum of some.

The orange perithecia place this fungus in the *Bionectriaceae* rather than in the *Nectriaceae*, where it is most closely aligned with *Bionectria* Speg. (Schroers, 2001), and its anamorph has characteristics which would place it in *Clonostachys* Corda, the anamorph of *Bionectria*. This fungus closely resembles *Bionectria epichloë* (Speg.) Schroers [syn. *Nectriopsis epichloë* (Speg.) Samuels], which has been found on the stromata of species of *Epichloë* and *Balansia* in the Americas (Samuels, 1988; Schroers, 2001). However, although the dimensions of the ascospores in the Australian specimens lie within the ranges for those of *N. epichloë,* its conidia are longer than those provided in Samuels' and Schroer's descriptions (5-8 x 2.5-5 µm and 4.8-9.6 x 2.2-3.6 µm, respectively). *Nectriopsis macroepichloë* Samuels was described as colonising ascostromata of *Balansia cyperacearum* (Berk. & M.A. Curtis) Diehl in Venezuela, but its ascospores are considerably longer than those of *B. epichloë* and the *Bionectri*a species on *N. scleroticus* ascostromata (Samuels, 1988). It is possible that the specimens of *Bionectria* found on some ascostromata of *N. scleroticus* in Australia represent a new species.

Acknowledgments

I thank Dr Ray Langdon, former Reader in Botany, University of Queensland, for his guidance and patience during my postgraduate studies, and for his esteemed and enduring friendship since then. I also thank the curators of herbaria for loans of type and other specimens listed in this paper. Thanks also to Dr Michael Priest, Dr Ian Pascoe, and Dr Shaun Pennycook for reviewing this paper.

Literature Cited

Arnold RH. 1967. The *Nectriella* element of '*Hyalodothis*'. Mycologia 59: 246-254.

Bailey FM. 1891. Contributions to the Queensland flora. Bot. Bull. Dep. Agric Qd 3.

Bailey FM. 1913. Comprehensive Catalogue of Queensland Plants. Government Printer, Brisbane.

Berkeley MJ, Curtis, MA. 1883. Sylloge Fungorum 2: 652.

Bischoff J.F., White JF Jr. 2003. The Plant-infecting Clavicipitaleans. *In* Clavicipitalean Fungi: Evolutionary Biology, Chemistry, Biocontrol, and Cultural Impacts (Eds. J White Jr, CW Bacon, NL Hywel-Jones, JW Spatafora), pp. 125-149. Marcel Dekker Inc., New York NY, USA.

Bresadola G, Saccardo PA. 1899. Fungi Congoenses enumerati I. Bull. Soc. Roy. Bot. Belg. 38: 161.

Brittlebank CC. 1940. The Brittlebank Catalogue of Fungi and Bacteria Causing Plant Diseases in Australia (unpublished, held in the National Herbarium of Victoria).

Castellani E, Ciferri R. 1937. Prod. Mycofl. Afr. Or. Ist. Agr. Coloniale Italiano, Firenze.

Cooke MC. 1892. Australian fungi. Grevillea 20: 4-7.

Diehl WW. 1950. *Balansia* and the *Balansiae* in America. Agriculture Monograph No. 4. United States Department of Agriculture, Washington DC.

Doidge EM. 1948. South African ascomycetes in the national herbarium. Part 4. Bothalia 4: 837-880.

Govindu HC, Thirumalachar MJ. 1963. Studies on some species of *Ephelis* and *Balansia* occurring in India. II Morphological and taxonomic status of *Balansia*. Mycopathol. Mycol. Appl. 20: 297-306.

Hennings P. 1895a. Fungi kamerunenses I. Bot. Jahrb. Syst. 22: 72-111.

Hennings P. 1895b. Pilze Ostafrikas in Deutsch-Ost Afrika. Band II. In Pflanzenw. Ost-Afrikas (Ed. A. Engler), Teil C, pp. 30-35. D. Reimer, Berlin.

Hennings P. 1900. Fungi in Zahlbrückner plantae pentherianae. Ann. Naturhist. Mus. Wien 15: 1-3.

Hennings P. 1901. Fungi japonici. Bot. Jahrb. Syst. 28: 274-275.

Hennings P. 1906. Fungi japonici VI. Bot. Jahrb. Syst. 37: 162.

Hodge KT. 2003. Clavicipitaceous anamorphs. In Clavicipitalean Fungi: Evolutionary Biology, Chemistry, Biocontrol, and Cultural Impacts (eds J White Jr, CW Bacon, NL Hywel-Jones, JW Spatafora), pp. 75-123. Marcel Dekker Inc., New York NY, USA.

Höhnel F Von. 1910. Fragmente zur Mykologie No. 630. Kaiserl. Akad. Wiss., Math.-Naturwiss. Kl., Abt. 1, 119: 57-65.

Höhnel F Von 1911. Fragmente zur Mykologie No. 704. Sitzungsber. Kaiserl. Akad. Wiss., Math.-Naturwiss. Kl., Abt. 1, 120: 449.

Luttrell ES, Bacon CW. 1977. Classification of *Myriogenospora* in the Clavicipitaceae. Can. J. Bot. 55: 2090-2097.

Massee G. 1896. New or critical fungi. J. Bot. 34: 145-154.

Palmer J, Lazarides M, McCusker A, Weiller CM. 2005. *Eragrostis*. In Flora of Australia Vol. 44B Poaceae 3 (ed K Mallett), pp. 346-409. ABRS/CSIRO Publishing, Melbourne, Australia.

Patouillard N. 1890. Contribution à la flore mycologique du Tonkin. VI. Pyrenomycetes. J. Bot. (Morot) 4: 61-67.

Patouillard N, Hariot P. 1893. Fungos aliquot novos in regione congoana collectos. Bull. Soc. mycol. Fr. 9: 206-211.

Petch T. 1916. Revisions of Ceylon fungi (Part IV). Ann. Roy. Bot. Gard. (Peradeniya) 6: 153-183.

Roger L. 1953. Phytopathologie des pays chauds. Tome II. Etude descriptive des Maladies parasitaires des plantes des pays chauds. Hypocréacées genre *Balansia* Speg. Encycl. mycol. 18: 1537.

Rossman A, Samuels GJ, Rogerson CT, Lowen R. 1999. Genera of *Bionectriaceae, Hypocreaceae* and *Nectriaceae* (*Hypocreales,* Ascomycetes). Studies in Mycology 42. Centraalbureau voor Schimmelcultures, Baarn/Delft, The Netherlands.

Ryley MJ. 2003. *Nigrocornus scleroticus,* a common Old World balansioid fungus. In Clavicipitalean Fungi: Evolutionary Biology, Chemistry, Biocontrol, and Cultural Impacts (eds J White Jr, CW Bacon, NL Hywel-Jones, JW Spatafora), pp. 247-271. Marcel Dekker Inc., New York NY, USA.

Samuels GJ. 1988. Fungicolous, Lichenicolous, and Myxomyceticolous Species of *Hypocreopsis, Nectriopsis, Nectria, Peristomialis,* and *Trichonectria*. Mem. New York Bot. Grdn. 48. Bronx, New York Botanical Garden.

Schroers H-J. 2001. A Monograph of *Bionectria* (Ascomycota, *Hypocreales, Bionectriaceae*) and its *Clonostachys* anamorph. Studies in Mycology 46. Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands.

Sydow H Von. 1935. Beschreibungen neuer sudafrikanischer pilze VI. Annls mycol. 33: 230-237.

Viennot-Bourgin G. 1964. Étude de micromycètes parasites récoltés a Madagascar, 3° Ascomycetes et Imperfecti. *Balansia madagascariensis* n.sp. Annls Inst. natn. agron., Paris, Ser. 2, 2: 16-25.

White JF Jr. 1997. Systematics of the graminicolous clavicipitaceae. In Neotyphodium/Grass Interactions (eds CW Bacon, NS Hill), pp. 27-39. Plenum Press, New York.

White JF Jr, Reddy PV. 1998. Examination of structure and molecular genetic phylogenetic relationships of some graminicolous symbionts in genera *Epichloë* and *Parepichloë*. Mycologia 90: 226-234.

MYCOTAXON

Volume 95, pp. 113–116 January–March 2006

Rick´s species revision: *Mitremyces zanchianus* versus *Calostoma zanchianum*

Iuri G. Baseia[1], Vagner G. Cortez[2] & Francisco D.Calonge[3]

baseia@cb.ufrn.br
[1]Universidade Federal do Rio Grande do Norte, CB
Depto. Botânica, Ecologia e Zoologia, 59072-970, Natal, RN, Brazil

cortezvg@yahoo.com.br
[2]Universidade Federal do Rio Grande do Sul
Depto. Botânica, 91501-970, Porto Alegre, RS, Brazil

calonge@ma-rjb.csic.es
[3]Real Jardín Botánico, CSIC
Plaza de Murillo 2, 28014 Madrid, Spain

Abstract — After the revision of *Mitremyces zanchianus* we confirm this species as a valid name in the *Sclerodermataceae* and propose a new combination to the genus *Calostoma*. Description and illustrations of the type and SEM images of the basidiospores are given.

Key words — *Agaricomycetidae, Sclerodermataceae,* Gasteromycetes, taxonomy, neotropics

Introduction

The role played by Rick in the development of Brazilian mycology is not measured by his numerous works alone (for a full list see Fidalgo, 1962), but by his collaboration with foreign mycologists (e.g. Lloyd, Rehm, Bresadola, Sydow), which furthered the study the Brazilian fungi. In addition, it is worth emphasizing that Rick influenced and encouraged fellow mycologists, such as Theissen and Torrend (Fidalgo 1962). A representative part of the Rick's collection in Brazil is preserved in the Herbarium Anchieta (PACA), located in the State of Rio Grande do Sul.

The genus *Calostoma,* which was established by Desvaux in 1809, has priority over *Mitremyces,* a synonym erected by Nees in 1817. According to Kirk et al. (2001), *Calostoma* comprises about 15 species and is currently included in the family *Sclerodermataceae* Corda (= *Calostomaceae* E. Fisch.). *Calostoma* species are characterized by the pseudostipitate basidioma with a peridium composed of two to four layers and opening by only one apical star-like pore, and basidiospores that are globose to elliptic with reticulate or pitted ornamentation (Miller & Miller 1988).

At present, no representatives of the *Calostoma* have been reported for Brazil except for *Mitremyces zanchianus,* a rare species described by Rick (1961, but see comments below). Our primary goal is to determine whether Rick's species should be placed in *Calostoma.*

Materials and methods

Macroscopic characters were examined following usual techniques utilized in taxonomic studies of gasteroid fungi including a detailed description from the fresh specimen. Microscopic characters were determined according to Miller & Miller (1988). Basidiospores were examined using a Philips XL20 Scanning Electron Microscope (SEM).

Results

Calostoma zanchianum (Rick) Baseia & Calonge, **nov. comb.** **Figs. 1-3**

Basionym: *Mitremyces zanchianus* Rick in Iheringia Sér. Bot..9: 456, 1961.

Basidiomata stalked, head egg-shaped, 1.3 cm long, 1 cm broad; exoperidium white silky, rather thick, gelatinous, rugose surface ruptured by the growth of fruitbody. Endoperidium pinkish white, cartilaginous, smooth. Mouth regularly star-shaped consisting of 4 long slits, splitting at maturity; stalk strongly dilated, subglobose, 1.2 cm long, 1.3 cm broad. Spores 30-35 x 15-20 μm, fusiform to elliptic, smooth, colourless to pale yellowish, provided with a longitudinal groove.

Material examined – *Holotypus* of Herbarium Anchieta (PACA 19.673), collected by Father Rômulo Zanchi in March 1943 from the current municipality of São João do Polesine (formerly Polesine, municipality of Cachoeira do Sul, as indicated in the herbarium notes), central part of Rio Grande do Sul State, Brazil.

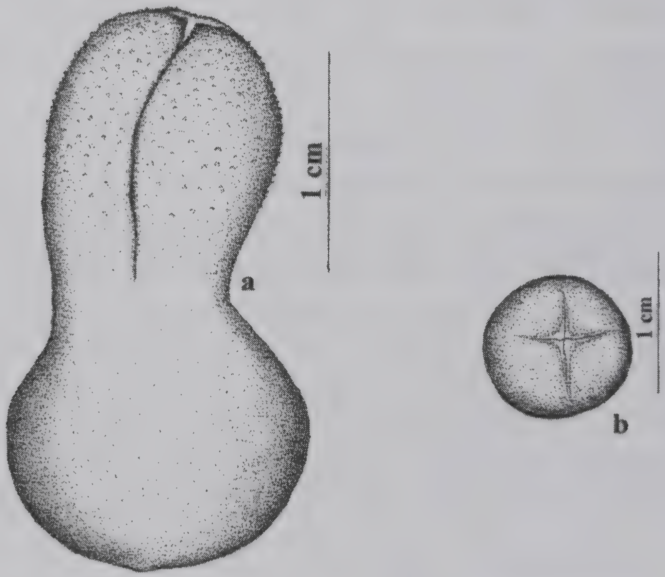


Fig. 1 *Calostoma zanchianum* (holotypus) **a**: Mature basidioma. **b**: Top view

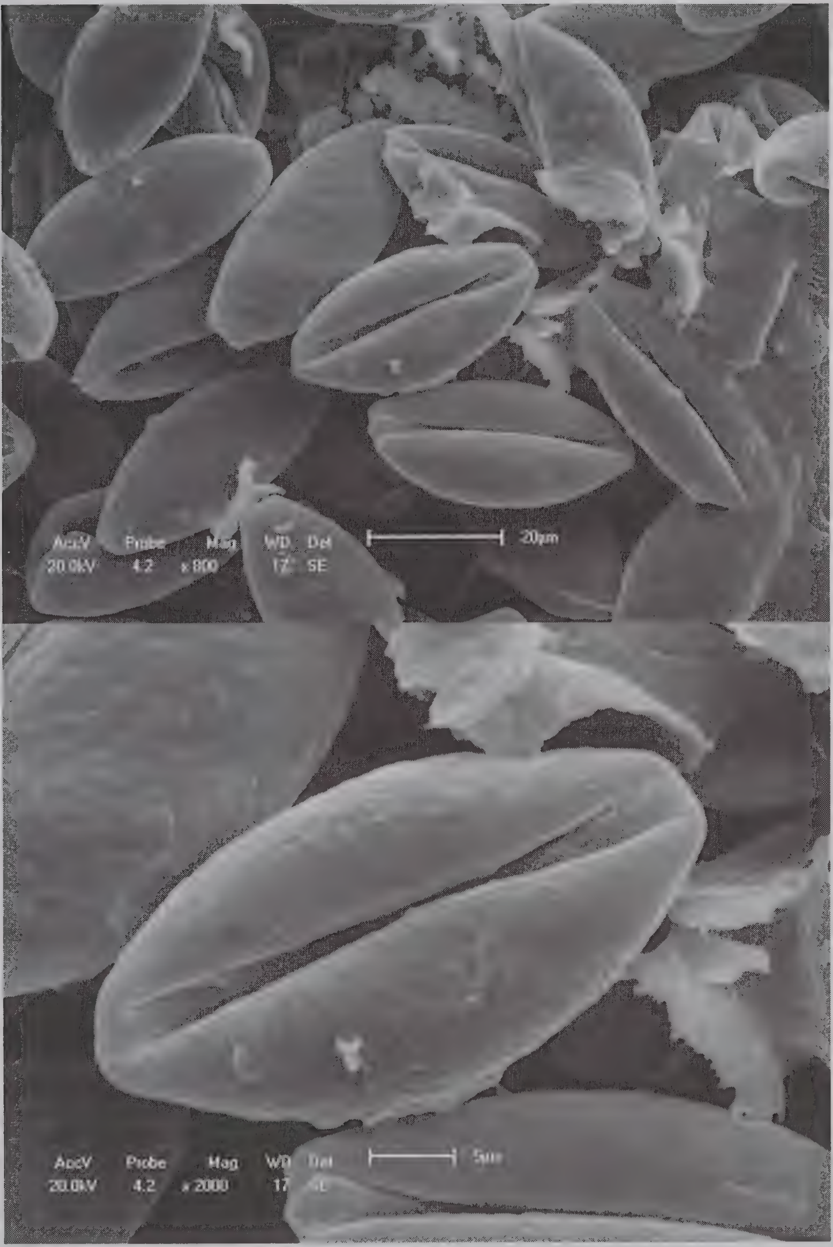

AccV Probe Mag WD Det
20.0kV 4.2 x 800 17 SE
20µm
AccV Probe Mag WD Det
20.0kV 4.2 x 2000 17 SE
5µm

Fig. 2-3: *Calostoma zanchianum* (holotypus). Basidiospores under SEM

Discussion

This species is easily recognized by several distinct characters: a stalk strongly dilated at the base, a mouth consisting of four long slits, and elliptic to fusiform basidiospores with a longitudinal groove. Those singular characters separate *Calostoma zanchianum* from other *Calostoma* species. This taxon also has some interesting ecological data: Zanchi's notes cite that it was found growing on roots of an unknown native tree.

In fact, Rick himself never published the collection as a new species. The species was published in a posthumous series (i.e., Basidiomycetes Eubasidii in Rio Grande do Sul – Brasilia) by Father Balduíno Rambo, another noteworthy botanist who compiled all fungi studied and collected by Rick, including several non-published taxa. Thus, *Mitremyces zanchianus* was only validly published 15 years after Rick's death in 1946.

Unfortunately, the only known material of this species is the holotype. Efforts are underway to collect new material so as to document the morphological variation and biology of this unusual species.

Acknowledgments

We express our sincere gratitude to the curator of Anchieta Herbarium (PACA) for the loaned material. Thanks are also given to the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for financial support; and to Tereza Cristina de Oliveira Galvão for the illustrations. We are grateful to Prof. H. Kreisel, Prof. G. Moreno and Dr. P. M. Kirk for the critical revision of the manuscript and checking of the English text.

References

Desvaux NA. 1809. Observations sur quelques genres à établir dans la famille dês Champignons. Journal de Botanique 2: 38-105.

Fidalgo O. 1962. Rick, o Pai da Micologia Brasileira. Rickia 1: 3-11.

Kirk PM, Cannon PF, David JC, Stalpers JA. 2001. Ainsworth & Bisby´s Dictionary of the Fungi. 9th ed. CABI Int. Publ., UK,

Miller OK, Miller HH. 1988. Gasteromycetes: Morphology and Developmental Features. Mad River, Eureka, CA, 156 pp.

Nees v. Essenbeck CG. 1817. Das System der Pilze und Schwämme. Würzburg, p. 136.

Rick J. 1961. Basidiomycetes Eubasidii in Rio Grande do Sul. Iheringia, Ser. Bot. 9: 451-490.

MYCOTAXON

Volume 95, pp. 117–131 January–March 2006

Taxonomic studies on Indian *Phellinus* s.l. species: parsimony analysis using morphological characters

Prasad Lamrood

pylamrood@yahoo.com
Department of Botany, Modern College
Ganeshkhind, Pune - 411 053, Maharashtra, India

Aristóteles Góes-Neto

agoesnt@uefs.br
Laboratório de Pesquisa em Microbiologia (LAPEM),
Depto. de Ciências Biológicas, Universidade Estadual de Feira de Santana (UEFS)
Km 3, BR-116 (norte), Feira de Santana, Bahia, 44.031-460, Brazil

Abstract — Parsimony analysis of Indian *Phellinus* s.l. species based on basidiomata morphological characters was carried out. Fifty-four samples belonging to 23 species collected from different hosts were used. Trees were produced using parsimony analysis, heuristics search with random taxon addition sequences, tree-bisection-reconnection branch swapping without topological constraints. Out of 84 morphological characters used in the present analysis, 35 characters were binary and 49 were multistate and treated as non-additive. Parsimony analysis revealed that most of the studied specimens could be assigned to *P. rimosus* complex (*Fulvifomes*), *P. pini* complex (*Porodaedalea*), and *P. igniarius* complex (*Phellinus* s.s.).

Key words — basidiomata morphology, cladistic analysis, *Hymenochaetaceae*, South Asia, tropical rain forest

Introduction

The circumscription of genera in the *Hymenochaetaceae* is imprecise, even in the field of traditional systematics (Corner, 1991). The same situation occurs at generic level, notably in *Phellinus* Quél., which accounts for more than half of the total number of species of Hymenochaetales. The genus *Phellinus* includes several species complexes, and is generally regarded as polyphyletic (Ryvarden 1991, Fischer 1996ab, Wagner & Fischer 2002). *Phellinus* s.l. is worldwide in distribution (Lloyd 1915, Bondarzew 1953, Overholts 1929, 1953, Lowe 1957, Cunningham 1965, Fidalgo 1968, Niemelä 1972, Ryvarden 1972, Donk 1974, Gilbertson & Ryvarden 1987, Rajchenberg 1989, Larsen & Cobb-Poulle 1990). A survey of the world taxa of *Phellinus* resulted in the recognition of 154 species and 67 forms and varieties (Larsen & Cobb-Poulle 1990, Rabba 1994, Fischer 1996ab).

Species of *Phellinus* are parasitic, perthophytic and/or saprobic causing white rot that degrades both lignin and cellulose (Rabba 1994, Vaidya 1995, Fischer 1996ab). They dwell on a wide variety of angiosperms and/or gymnosperms (Wagner & Fischer 2002) causing heart rot disease in live standing trees. Besides being a wood decaying fungus, *Phellinus* serves as a medicine in the western part of India (Vaidya & Bhor 1991, Vaidya & Rabba 1993, Vaidya & Lamrood 2000) and also in traditional Chinese and Korean medicine (Mizuno 2000, Smith et al. 2002).

Species are characterized by poroid basidiomata with variable shape that even between closely related species may range from resupinate, effused-reflexed, pileate, and substipitate to stipitate (Wagner & Fischer 2002). The generic concepts of *Phellinus* and its closest relative *Inonotus* P. Karst. have been traditionally based on mitism of the hyphal system and consistency of the fruit body (i.e. *Phellinus*: dimitic and perennial; *Inonotus*: monomitic and annual) but this has been repeatedly demonstrated as artificial (Fiasson & Niemelä 1984, Corner 1991, Dai 1999, Fischer 1996ab, Wagner & Fischer 2002), and inconsistent in *Phellinus* (Fischer 1996ab). In several taxa, the dimitic hyphal system is vague or lacking, e.g. members of *P. robustus* complex and *P. sulphurascens* Pilát (Fischer 1996b). Intermediate forms occur as in *P. discipes* (Berk.) Ryvarden, *P. erectus* A. David et al., and *P. gilvus* (Schwein.) Pat., in which the fruit bodies are annual and have dimitic hyphal system. On the other hand, species like *P. pachyphloeus* (Pat.) Pat., *P. poeltii* Ryvarden, *P. robustus* (P. Karst.) Bourdot & Galzin and *P. sulphurascens* have perennial fruit bodies but have monomitic hyphal system or show transitions between monomitic and dimitic hyphal systems (Domanski et al. 1973, Jahn 1981, Fiasson & Niemelä 1984, Corner 1991, Ryvarden & Gilbertson 1994, Wagner & Fischer 2002).

Extensive studies on the occurrence and systematics of *Phellinus* have been carried out for European and North American taxa (Jahn 1981, Ryvarden 1978, Fiasson & Niemelä 1984, Jülich 1984, Gilbertson & Ryvarden, 1986, 1987, Ryvarden & Gilbertson, 1994). As stated by numerous authors (Bondarzew 1953, Niemelä 1972, 1975, Domanski et al. 1973, Kotlaba & Pouzar 1978, Ryvarden 1978, Fiasson & Niemelä 1984, Parmasto 1988, Ryvarden & Gilbertson 1994, Fischer 1996b) the European taxa of *Phellinus* can be divided into a number of groups. Besides the type species, *Phellinus* contains a varying number of closely related taxa, which, in many cases, were originally considered as varieties or forms of the type species. The species complexes that are recognized for Europe are: *P. igniarius* complex, *P. pini* complex (Fischer 1996ab), and *P. robustus* complex. Three additional groups with *P. ferrugineofuscus* (P. Karst.) Bourdot & Galzin, *P. ferruginosus* (Schrad.) Pat., and *P. rimosus* (Berk.) Pilát are also recognized (Fischer 1996b). The *P. igniarius* and *P. robustus* complexes are also mentioned for North America (Gilbertson 1979).

Numerous characters from morphology, anatomy, sexuality, nuclear behaviour, pigmentation and ecology would suggest that *Phellinus* is heterogeneous (Fiasson 1982, Fiasson & Niemelä 1984, Dai 1999, Fischer 1996b). Because of the uncertainty of the concept of *Phellinus*, as well as recognized divergences within the genus, the species complexes have repeatedly been acknowledged as separate genera. Based upon the examination of mostly North American and European collections, several attempts were made to split *Phellinus* into smaller, more natural genera. As a result *Phellinus* s.l. comprises numerous generic notions (Fiasson & Niemelä 1984, Wagner & Fischer 2002) made up of *Fomitiporella* Murrill, *Fomitiporia* Murrill, *Fulvifomes* Murrill, *Fuscoporella* Murrill, *Fuscoporia* Murrill, *Ochroporus* J. Schröt., *Phaeoporus* J. Schröt., *Phellinidium* (Kotl.) Fiasson & Niemelä, *Phellopilus* Niemelä et al., *Phylloporia* Murrill, *Porodaedalea* Murrill, *Pseudofomes* Lázaro Ibiza, *Pyropolyporus* Murrill, and *Scalaria* Lázaro Ibiza (Wagner & Fischer 2002).

The comprehensive study of Fiasson & Niemelä (1984) was mostly based on morphology, anatomy, pigmentation, protein pattern, cultural type and nuclear behaviour. Based on the characters derived from this study, Fiasson & Niemelä (1984) revised the taxonomy of the European poroid Hymenchaetales and proposed to split the Hymenchaetales into two families, Phellinaceae, composed of *Fomitiporia*, *Fulvifomes*, *Fuscoporia*, *Inonotopsis* Parmasto, *Ochroporus*, *Onnia* P. Karst., *Phellinidium*, *Phellinus* s.s., and *Porodaedalea*, and a newly described Inonotaceae, composed of *Inonotus* s.s., *Inocutis* Fiasson & Niemelä, and *Phylloporia* (Wagner & Fischer 2002). Since the study of Fiasson & Niemelä was mostly based on European material, neglecting the numerous tropical species, this suggested splitting concept was not followed subsequently (Gilbertson & Ryvarden 1986, 1987, Parmasto 1988, Larsen & Cobb-Poulle 1990, Ryvarden & Gilbertson 1994). The generic splitting has been accepted in only a few studies such as Nuss (1986) and Hansen & Knudsen (1997). Dai (1999) also accepted genera like *Fomitiporia* and *Phellinidium* but granted subgeneric level to *Fulvifomes*, *Fuscoporia*, *Phellinidiopsis*, *Phellinus* s.s., and *Porodaedalea* (Wagner & Fischer 2002).

As extensive phylogenetic data are still missing for non-European taxa, which make up approximately two thirds of the presently known species, the generic concept of *Phellinus* is mostly based on the European taxa (Wagner & Fischer 2002). However, fragmentary work has been done in India, mainly pertaining to Himalaya and a few parts of the central Peninsula (Bagchee & Bakshi 1950, Bagchee 1961, Singh 1966, Bakshi 1958, 1971, Thind & Chatrath 1957, Thind & Dhanda 1980, Roy 1979, Tiwari et al. 1989, Sharma, 1993), as well as in southern parts (Ganesh & Leelavathy 1986, Natarajan & Kolanduvelu 1985). The most comprehensive account on this genus in Maharashtra is the work of Rabba (1994). Before Rabba (1994) and Rabba et al. (1994) only nine species were known from Maharashtra and 50 species from India (as *Fomes*). High rainfall, high humidity and hot temperature favour the growth of many species of *Phellinus* in the forests of Maharashtra. Neither the species (Parmasto 1985) nor the generic concepts in *Phellinus* are stable and will only become more concise when species from all parts of the globe are included in an analysis of the genus (Wagner & Fischer 2002). Towards this end, a study was undertaken of *Phellinus* s.l. found in Maharashtra.

Materials and methods

Collection of samples

Fifty-four samples of *Phellinus* were collected in India, mainly during the monsoon season. Six samples were collected in 1987-92 while the other samples were collected during 1998-2002. Collections were made in various regions of western Maharashtra (i.e. Konkan region), including in and around Pune city (Pune University Campus (PUC), Amba valley, and Simhagarh fort) on various plant hosts (Table 1).

The samples were kept in clean polythene bags with a label indicating the date and place of collection, name of the collector, name of the host (in case of field collection), name of the shop and shop owner (in case of market collection), type of fruiting body and a brief description of the specimen. Specimens were then taken to the laboratory.

Spore prints, obtained from fresh basidiomata on cellophane paper, were maintained in airtight plastic bags. After drying in a oven at 40°-45°C the basidiomata were stored in cardboard boxes and deposited in Herbaria Poonensis, Department of Botany, University of Pune under the accession number 'PH'. All the characters used in the analysis were evaluated in dried basidiomata.

Identification

Specimens were identified following a study of external and internal morphology. The color, texture, type of attachment of the basidiomata, hymenial and pileal surface of the basidiomata, margin, pore morphology, and dissepiment were observed and noted. Measurements of basidiomata were taken as in Ryvarden & Johansen (1980). Detailed microscopic examinations were carried out by cutting freehand longitudinal thin sections of basidiomata, passing through hymenia. Sections were first treated with absolute alcohol for a few seconds and then transferred to a solution of 10% KOH to allow the swelling of different hymenial structures. Sections were washed two to three times with distilled water (1 minute per washing), then stained with 1% phloxine (for visualization of basidia), and teased apart in lactoglycerine with 1% cotton blue (for visualization of setae, hyphal tips, hyphae, etc.). Semipermanent slides were prepared in lactoglycerine. Permanent slides were prepared in polyvinyl alcohol (PVA) medium (Omar et al. 1979) and observed under a bright field microscope (Olympus BX 40) with an attached color camera (Hamamatsu 3CCD C6157 and UVP).

The identification of specimens was done using a key (Larsen & Cobb-Poulle 1990). The color scheme of Jordan & David (1995) was used to describe color of morphological structures.

Morphological Characters

Eighty-four morphological characters were used in the analysis. The list of characters and character states (Table 2) was prepared according to Góes-Neto et al. (2001) and Kim & Jung (2002). A character state matrix was then prepared accordingly.

The characters of *Phellinus merrillii* and *P. linteus* were compared with the descriptions given by Larsen & Cobb-Poulle (1990), Rabba (1994), Dai & Xu (1998) and Lim et al. (2003), abbreviated as LCP, R, D, Y, respectively. Similarly, the description of *P. baumii* Pilát was compared with those in Larsen & Cobb-Poulle (1990), Dai & Xu (1998) and Lim et al. (2003).

Parsimony analysis

The character code for any character 'not determined' was coded as (?) and any character that was absent in a particular species was coded as missing (–). The newly generated data matrix was analyzed with PAUP 4.0 (Swofford 1999). Unrooted trees were produced using parsimony analysis, heuristics search with random taxon addition sequences, tree-bisection-reconnection branch swapping without topological constraints.

Results

Fifty-four samples belonging to 23 species were collected from 18 different plant hosts. Parsimony analysis conducted in PAUP resulted in 12 most parsimonious trees of 291 steps in length with CI = 0.38 and RI = 0.43. The strict consensus tree is represented in Fig. 1. Three retrieved clades are consistent with proposed *Phellinus* species complexes (or genera).

Clade 1 comprised *P. torulosus*, *P. baumii* (LCP), *P. conchatus*, *P. baumii* (D), *P. baumii* (Y), *P. linteus* (D), *P. linteus* (Y), *P. linteus*, *P. linteus* (LCP), and *P. linteus* (R), species that have hymenial setae, hyaline to brown spores, pileate basidiomata, and skeletal hyphae that are brown, thick-walled, septate, and rarely branched.

Furthermore, *P. baumii* (D) and *P. baumii* (Y) are closer to each other than to *P. baumii* (LCP), which grouped with *P. conchatus*. These two taxa are similar with respect to many characters but have distinct setal characters. *P. baumii* (LCP) has only hymenial setae while *P. conchatus* has both hymenial and tramal setae. These taxa have hyaline spores while most of the characters of generative and skeletal hyphae are missing in the case of *P. baumii* (LCP). Although *P. baumii* is usually treated as akin to *P. linteus*, it was observed that *P. linteus* forms a less inclusive clade sharing characters like presence of hymenial setae and brown spores, but the spores of *P. linteus* are slightly thick-walled. Also, the spores of *P. baumii* are ellipsoid while *P. linteus* has ovoid spores. Moreover, *P. linteus* as described by Dai & Xu (1998) and Lim et al. (2003) was not similar to the *P. linteus* described by Larsen & Cobb-Poulle (1990), Rabba (1994), and the sample of the present study. This could be attributed to the fact that many characters are missing.

Clade 2 is composed of *P. rickii*, *P.* sp., *P. lamaensis* and *P. orientalis*. Although all these taxa have subglobose, thin-walled and hyaline basidiospores, the former two have only hymenial setae while the latter two have tramal and hymenial setae.

Clade 3 is formed by the species *P. coffeatoporus*, *P. pappianus*, *P. badius*, *P. macgregorii*, *P. stratosus*, *P. fastuosus*, *P. lloydii*, *P. merrillii* (R), *P. merrillii* and *P. merrillii* (LCP). All these species lack setae, and have slightly thick or thick-walled, brown basidiospores.

Discussion

This is the first attempt to analyze the species concept of Indian species of *Phellinus*. In this study, character evolution was not considered and the study was entirely based on the morphological characters.

Because of the complexity in the family as well as in the genera of *Hymenochaetaceae*, mycologists have divided the family and even genera into smaller taxa. This has resulted in several species complexes especially within the genus *Phellinus*, e.g. *P. rimosus* complex, *P. pini* complex, *P. igniarius* complex, etc. The parsimony analysis revealed that many of the studied specimens could be assigned to at least three of these complexes.

P. conchatus, for example, was previously regarded as a member of *P. pini* complex. Fiasson & Niemelä (1984) proposed a new combination for this species using *Porodaedalea* as a generic name, i.e. *Porodaedalea conchata* instead of *Phellinus conchatus*. *P. conchatus*, however, was regarded as a member of *P. igniarius* complex by Fischer (1987). In the present study *P. conchatus* grouped together with *P. baumii* (LCP).

However, *P. baumii* was treated as a close member of *P. linteus*. In addition, *P. torulosus* also grouped together with these species.

P. igniarius complex is recognized by the species having well developed dimitic hyphal system, setae always present, and non-dextrinoid and hyaline basidiospores becoming brown or yellow with age. Both *P. conchatus* and *P. baumii* (LCP) comply with the characters of this complex but *P. conchatus* has both tramal and hymenial setae while *P. baumii* has only hymenial setae. Moreover, *P. conchatus* is oligonucleate, haploid, and bipolar heterothallic while *P. torulosus* is binucleate (Fischer 1996b). Furthermore, *P. torulosus* shares characters of several complexes within *Phellinus* and seems to be closer to *P. ferreus* and *P. pini* (Fiasson and Niemelä 1984). The taxonomic position of *P. torulosus* is rather isolated in Europe and more closely related to taxa occurring in North America (*P. gilvus*) or the tropics (*P. licnoides* (Mont.) Pat.). In the present study *P. torulosus* shares characters of *P. iginarius* complex along with *P. conchatus* and *P. baumii* (LCP).

P. baumii and *P. linteus* can be distinguished on morphological features. *P. baumii* has long been regarded as a synonym of *P. linteus*, owing to morphological similarities, until Dai & Xu (1998) discriminated them (Lim et al. 2003). These species are well separated from each other in Clade 1. Moreover, although *P. baumii* is closer to *P. linteus*, it grouped with other *Phellinus* spp. of a major '*P. igniarius* complex' clade. The Indian specimen of *P. linteus*, *P. linteus* (LCP) and *P. linteus* (R) were closer to each other than to *P. linteus* (D) and *P. linteus* (Y). It is suggested that *P. linteus* might have intermediate forms and these taxa, especially those from India, need further evaluation using other data sets.

Taxa of the *P. pini* complex are apparently restricted to Europe (Fischer 1996a). However, Overholts (1953), Owens (1936), and Parmasto (1985), based on morphology, suggested the existence of undescribed taxa within *P. pini* complex.

The *P. pini* complex is characterized by species with dimitic hyphal system with skeletal hyphae, annual or perennial basidiomata, presence of setae, and basidiospores with variable morphology that are hyaline, becoming slightly yellow with age, and nondextrinoid. The basidiospores and host specificity to conifers distinguishes the *P. pini* complex from other groups. In the present analysis, taxa like *P. rickii*, *P.* sp. (probably a new species), *P. lamaensis* and *P. orientalis* were observed to share the characters of the complex. *P.* sp., *P. lamaensis* and *P. orientalis* have resupinate to effused-reflex basidiomata. Amongst these three species, *P.* sp. has annual basidiomata and host specificity. It was found only on *Broussonetia papyrifera*, while only *P. rickii* has pileate, applanate basidiomata. In basidiospore morphology, all species of this clade except for *P. rickii*, have hyaline, thin-walled, subglobose basidiospores; *P. rickii* has brown thin-walled spores. Both hymenial and tramal setae were observed in *P. lamaensis* and *P. orientalis*, and only hymenial setae were observed in *P. rickii* and *P.* sp.

The *P. rimosus* complex has been recognized as a unique group with similar rusty brown spores, absence of setae, and ungulate basidiomata rapidly becoming black and rimose. Murrill proposed a new name *Fulvifomes* Murrill 1914, for this group. Ryvarden (1987) included *P. badius*, *P. caryophylii*, *P. fastuosus*, *P. nilgheriensis*, *P. robiniae* and *P. rimosus* in this complex. During the present study it was observed that a large clade shares these characters. Along with *P. badius* and *P. fastuosus*, species like *P. coffeatoporus*, *P. pappianus*, *P. macgregorii*, *P. stratosus*, *P. lloydii*, *P. merrillii* (R), *P. merrillii* (LCP), and *P. merrillii* (specimens of present study) agree with the characters of *P. rimosus* complex.

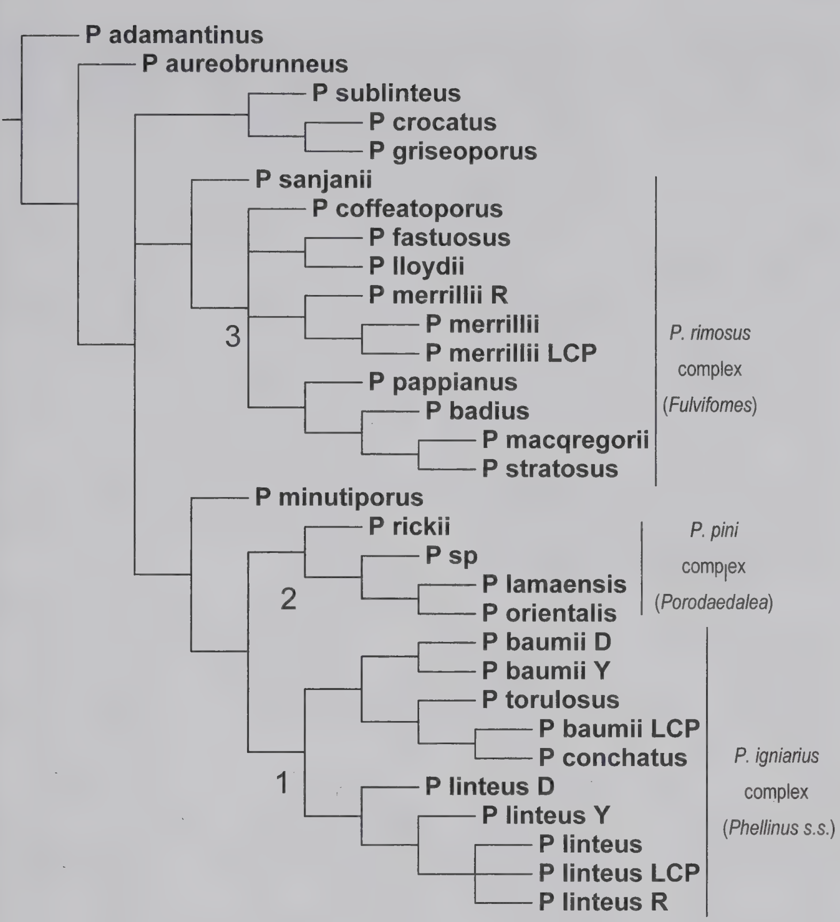

P adamantinus
P aureobrunneus
P sublinteus
P crocatus
P griseoporus
P sanjanii
P coffeatoporus
P fastuosus
P lloydii
P merrillii R
P merrillii
P merrillii LCP
P pappianus
P badius
P macqregorii
P stratosus
3
P. rimosus complex (Fulvifomes)
P minutiporus
P rickii
P sp
P lamaensis
P orientalis
2
P. pini complex (Porodaedalea)
P baumii D
P baumii Y
P torulosus
P baumii LCP
P conchatus
P linteus D
P linteus Y
P linteus
P linteus LCP
P linteus R
1
P. igniarius complex (Phellinus s.s.)

Fig. 1. Strict consensus tree for 23 *Phellinus* species in India.
Note: (Description of species according to LCP = Larsen and Cobb- Poulle 1990, D = Dai and Xu 1998, R = Rabba 1994, Y = Y. Lim et al. 2003.).

These species have pigmented brown spores, lack setae, and have pileate, perennial basidiomata.

P. sublinteus, *P. griseoporus*, and *P. crocatus* have varying basidioma morphology, and basidiospore shape and color. All, however, have thick-walled basidiospores. This clade was observed to be somewhat isolated from the rest of the main clades and the species within the clade could not be assigned to any particular species complex, and thus need further evaluation.

P. minutiporus showed rather close association with the taxa belonging to *P. iginarius* complex while *P. sanjanii* were closer to *P. rimosus* complex than the other studied taxa. Both *P. adamantinus* and *P. aureobrunneus* formed residual taxa in the tree and could not be assigned to any particular species complex.

Table 1: *Phellinus* collections studied and their collection data.

Species	Host	Site	Date	Sample
P. adamantinus (Berk.) Ryvarden	*Albizia lebbeck* (L.) Benth.	Near Kothi Gate (PUC)	2.VIII.2000	PH5A
	Eucalyptus globulus Labill.	Alice Garden (PUC)	27.VII.2000	PH3
	Tamarindus indica L.	Bremen Square, Aundh, Pune	5.VI.2001	PH20
P. aureobrunneus J.E. Wright & Blumenf.	*Albizia lebbeck*	Near Kothi Gate (PUC)	2.VIII.2000	PH4
P. badius (Berk.) G. Cunn.	*Acacia nilotica* (L) Delile	Lake side of Pashan	VI.1999	PH12
P. coffeatoporus Kotl. & Pouzar	*Acacia nilotica*	In front of Botany Department (PUC)	2.VIII.2000	PH13
P. conchatus (Pers.) Quél.	*Actinodaphne angustifolia* (Blume) Nees	Bhimashankar	27.IX.1987	VA-252
P. crocatus (Fr.) Ryvarden	*Acacia nilotica*	Alice Garden (PUC)	21.VII.2000	PH7
P. fastuosus (Lév.) Ryvarden	*Artocarpus integrifolius* L.f.	Sandu Brothers, Nerul, Navi Mumbai	III.2000	PH34S1
		Adali, Sawantwadi, Sindhudurg	VII.1988	Adali20
P. griseoporus D.A. Reid	*Albizia lebbeck*	Near Kothi Gate (PUC)	2.VIII.2000	PH5
		Near Kothi Gate (PUC)	2.VIII.2000	PH6
P. lamaensis (Murrill) Sacc. & Trotter	*Olea dioica* Roxb.	Bhimashankar	25.XII.2001	PH41
P. linteus (Berk. & M.A. Curtis) Teng.	*Mangifera indica* L.	Amba Valley	25.XII.2000	PH24
	Olea dioica	Karnala Bird Sanctury	19.IX.2000	PH30
	Pongamia glabra Vent.	Karnala Bird Sanctury	19.IX.2000	PH29
	Tamarindus indica	Alibaugh	14.IX.2000	PH18
P. lloydii (Cleland) G. Cunn.	*Artocarpus integrifolius*	Base of Sinhagad Fort	1.VII.2000	PH9
		Seetabai cha Dara Sinhagad Fort	1.VII.2000	PH9A
		Lokhande Aushadhalaya (LA), Ravivar Peth, Pune	IV.1999	PH33L2
P. merrillii (Murrill) Ryvarden	*Acacia leucophloea* Willd.	Anthropology Dept. (PUC)	17.VII.2000	PH15
		Anthropology Dept. (PUC)	17.VII.2000	PH15A
	Acacia nilotica	Lake side of Pashan	13.VIII.2001	PH17
	Artocarpus integrifolius	Lokhande Aushadhalaya, (LA), Ravivar Peth, Pune	IV.1999	PH32L1
		Sandu Brothers, Nerul, Navi Mumbai	III.2000	PH35S2
		Bhakti Cha Mala, Dapoli	IX.1998	PH36B1
		Bhakti Cha Mala, Dapoli	IX.1998	PH37B2

Table 1. (cont.).

Species	Host	Site	Date	Sample
P. merrillii (cont.)	*Artocarpus integrifolius* (cont.)	Nardave, Kankavali, Sindhudurg	XII.2001	PH47A
		Nardave, Kankavali, Sindhudurg	XII.2001	PH47B
		Nardave, Kankavali, Sindhudurg	XII.2001	PH47C
		Nardave, Kankavali, Sindhudurg	XII.2001	PH47D
		Adali, Sawantwadi, Sindhudurg	VII.1988	Adali21
		Deorukh, Ratnagiri	V.2002	M2
		Deorukh, Ratnagiri	V.2002	M4
	Azadirachta indica A. Juss.	Near Arts Faculty (PUC)	15.VII.2000	PH10
	Caesalpinia coriaria (Jacq.) Willd.	Near Examination Section (PUC)	15.VII.2000	PH16
	Gliricidia sepium (Jacq.) Kunth ex Walp.	Pune University Campus (PUC)	2.VIII.2000	PH2
		Near Post Office (PUC)	21.VII.2000	PH8
		Near S.T.P. (PUC)	2.VIII.2000	PH43
		Near S.T.P. (PUC)	15.VIII.2000	PH21
P. macgregorii (Bres.) Ryvarden	*Acacia nilotica*	Pune University Campus	26.XI.1992	VA-117
P. minutiporus Bondartseva & S. Herrera	*Albizia lebbeck*	Aundh Gate, (PUC)	2.VIII. 2000	PH31
P. orientalis Bondartseva & S. Herrera	*Thespesia populnea* (L.) Sol. ex Correa	Harihareshwar	19.IX.2000	PH1
P. pappianus (Bres.) Ryvarden	*Acacia nilotica*	Near Examination Section	2.VIII.2000	PH22
P. rickii Teixeira	*Swietenia mahagoni* (L.) Jacq.	Alice Garden (PUC)	17.VII.2000	PH14
P. sanjanii (Lloyd) Ryvarden	*Tamarindus indica*	Pune University Campus (PUC)	24.IX.1988	VA-354
P. stratosus Pat.	*Tamarindus indica*	Pune University Campus (PUC)	15.III.1992	VA-287
P. sublinteus (Murrill) Ryvarden	*Azadirachta indica*	Between S.T.P. and E.M.R.C. (PUC)	2.VIII.2000	PH23
	Gliricidia sepium	Between S.T.P. and E.M.R.C. (PUC)	2.VIII.2000	PH19
	Mangifera indica	Radhanagari	XI.2000	PH42
	Peltophorum ferrugineum Benth.	Alice Garden (PUC)	27.VII.2000	PH25
		Rear of Main Building (PUC)	IX.1999	PH26
P. torulosus (Pers.) Bourdot & Galzin	*Cassia fistula* L.	Central Bee Research Station, Pune	VI.2000	PH27
Phellinus sp.	*Broussonetia papyrifera* (L.) Vent.	Botanical Garden, Botany Dept. (PUC)	VI.2002	PH44

Table 2. List of characters and character states.

Character No.	CHARACTER	Character State
1	Life strategy	0 = annual, 1 = perennial
2	Basidioma morphology	0 = resupinate, 1 = effused-reflex, 2 = pileate
3	Basidioma	0 = sessile, 1 = spurious stipitate
4	Type of basidioma	0 = confluent, 1 = applanate, 2 = ungulate, 3 = imbricate, 4 = triquetrous, 5 = bracket, 6 = semi ungulate, 7 = pendent, 8 = convex
5	Attachment of pilei	0 = semicircular, 1 = dimidiate, 2 = lobate
6	Base of pilei	0 = attached by broad base, 1 = attached by narrow base, 2 = attached laterally
7	Consistency of basidioma	0 = brittle, 1 = corky, 2 = woody hard, 3 = pulvinate
8	Weight of basidioma	0 = light, 1 = medium, 2 = heavy
9	Separability from host	0 = easily, 1 = not easily
10	Basidioma gregariousness	0 = solitary, 1 = grouped
11	Color of pileal surface	0 = yellow, 1 = brown, 2 = red, 3 = black
12	Texture of pileal surface	0 = glabrous, 1 = velvety, 2 = tomentose, 3 = velutinate, 4 = pruinose
13	Zonation of pileal surface	0 = azonate, 1 = zonate
14	Sulcation of pileal surface	0 = sulcate, 1 = non sulcate
15	Cracks in pileal surface	0 = cracking, 1 = not cracking
16	Warts in pileal surface	0 = warty, 1 = not warty
17	Rimosity of pileal surface	0 = rimose, 1 = not rimose
18	Nodosity of pileal surface	0 = nodose, 1 = not nodose
19	Rugosity of pileal surface	0 = rugose, 1 = not rugose
20	Ridges in pileal surface	0 = ridged, 1 = not ridged
21	Induration of pileal surface	0 = indurate, 1 = not indurate
22	Shape of pileal surface	0 = flat, 1 = rounded
23	Moss covered	0 = yes, 1 = no
24	Margin	0 = entire, 1 = lobed
25	Thickness of margin	0 = thin, 1 = thick
26	Shape of margin	0 = acute, 1 = obtuse, 2 = blunt, 3 = rounded, 4 = nodular
27	Fertility of margin	0 = sterile, 1 = fertile
28	Texture of margin	0 = glabrous, 1 = tomentose, 2 = velutinate
29	Color of margin	0 = yellow, 1 = brown
30	Color of margin compared to pore surface	0 = paler than pore surface, 1 = darker than pore surface, 2 = concolorous
31	Consistency of margin	0 = brittle, 1 = firm
32	Morphology of margin	0 = soft, 1 = rough, 2 = sulcate
33	Size of margin	0 = below 2.5 mm, 1 = above 2.5 mm, 2 = more that 5 mm
34	Color of pore surface	0 = yellow, 1 = brown, 2 = red, 3 = gray

Table 2. (cont.).

Character No.	CHARACTER	Character State
35	Pore surface in light	0 = not glancing, 1 = glancing
36	Shape of pore surface	0 = flat, 1 = round
37	Texture of pore surface	0 = glabrous, 1 = tomentose, 2 = sulcate
38	Pore shape	0 = angular, 1 = circular, 2 = irregular, 3 = regular, 4 = cappilate and / or toothed
39	Pores per mm	0 = up to 6, 1 = up to 9, 2 = up to 12
40	Tube layer	0 = indistinctly stratified, 1 = distinctly stratified
41	Color of tubes	0 = yellow, 1 = brown, 2 = red
42	Color of tube layer	0 = paler than context, 1 = concolorous, 2 = darker than context
43	Length of tubes	0 = short, 1 = long
44	Layer strata	0 = single, 1 = double or multiple
45	Black line between layers	0 = absent, 1 = present
46	Size of tubes	0 = up to 5 mm, 1 = up to 7 mm, 2 = up to 10 mm, 3 = up to 12 mm, 4 = more than 12 mm
47	Thickness of context	0 = thin, 1 = thick
48	Consistency of context	0 = fibrous, 1 = corky, 2 = woody hard
49	Luster of context	0 = silky or golden on broken surfaces, 1 = without a luster, 2 = fibrous luster
50	Texture of context	0 = azonate, 1 = zonate
51	Dark line between context	0 = absent, 1 = present
52	Tissue of context	0 = of one kind, 1 = of two kinds
53	Color of context	0 = yellow, 1 = brown, 2 = red, 3 = black
54	Size of context	0 = up to 5 mm, 1 = up to 10 mm, 2 = more than 10 mm
55	Generative hyphae	0 = thin-walled, 1 = thick-walled
56	Branching of generative hyphae	0 = branched, 1 = unbranched, 2 = rarely branched
57	Septa of generative hyphae	0 = unseptate, 1 = rarely septate, 2 = septate
58	Color of generative hyphae	0 = hyaline, 1 = yellow, 2 = brown, 3 = green
59	Size of generative hyphae	0 = up to 4 μm, 1 = up to 5 μm
60	Skeletal hyphae	0 = thin-walled, 1 = thick-walled
61	Branching of skeletal hyphae	0 = branched, 1 = unbranched, 2 = rarely branched
62	Septa of skeletal hyphae	0 = non-septate, 1 = rarely septate, 2 = septate
63	Color of skeletal hyphae	0 = yellow, 1 = brown
64	Size of skeletal hyphae	0 = below 6 μm, 1 = above 6 μm
65	Setae	0 = both absent, 1 = only hymenial setae, 2 = both present
66	Hymenial setae	0 = thick-walled, 1 = thin-walled, 2 = absent
67	Shape of hymenial setae	0 = straight, 1 = ventricose, 2 = uncinate, 3 = subulate, 4 = fusiform, 5 = conical, 6 = absent

Table 2 (*concluded*)

Character No.	CHARACTER	Character State
68	Septa of hymenial setae	0 = non-septate, 1 = septate, 2 = absent
69	Base of hymenial setae	0 = globular base, 1 = ventricose, 2 = inflated, 3 = bent base, 4 = bulbous base, 5 = absent
70	Tip of hymenial setae	0 = ventricose, 1 = straight, 2 = acute, 3 = absent
71	Color of hymenial setae in water	0 = yellow, 1 = brown, 2 = absent
72	Color of hymenial setae in KOH	0 = yellow, 1 = brown, 2 = absent
73	Tramal setae	0 = thick-walled, 1 = thin-walled, 2 = very elongated, 3 = absent
74	Septa of tramal setae	0 = non-septate, 1 = septate, 2 = absent
75	Apex of tramal setae	0 = acute, 1 = blunt, 2 = absent
76	Shape of tramal setae	0 = cylindrical, 1 = elongated, 2 = absent
77	Color of tramal setae in water	0 = yellow, 1 = brown, 2 = absent
78	Color of tramal setae in KOH	0 = yellow, 1 = brown, 2 = absent
79	Basidium shape	0 = globose, 1 = subglobose, 2 = clavate, 3 = pyriform, 4 = barrel shaped
80	Basidiospore morphology	0 = globose, 1 = subglobose, 2 = ovoid, 3 = ellipsoid
81	Basidiospore wall	0 = thin, 1 = thick, 2 = slightly thick
82	Basidiospore pigmentation	0 = hyaline, 1 = pigmented
83	Basidiospore color in water	0 = yellow, 1 = brown, 2 = hyaline
84	Basidiospore color in KOH	0 = pale golden brown, 1 = dull brown, 2 = olivaceous, 3 = brown

Acknowledgements

We would like to thank all the people that indirectly or directly contributed to this work, and, especially, Dr James Ginns and Dr Eric McKenzie for exhaustive reviews and invaluable criticism. This work is part of the PhD thesis of the first author and he would like to thank Dr Jitendra Vaidya as well as the head of the Department of Botany, University of Pune for providing necessary facilities.

Literature cited

Bagchee KD. 1961. The fungal diseases of Sal (*Shorea robusta* Gaertn. F. and Fils) IV. *Fomes caryophyllii* (Rac.) Bres. a destructive heart rot of Sal. Indian Forest Records (N.S.) 2: 25-58.

Bagchee KD, Bakshi BK. 1950. Some fungi as wound parasite on Indian trees. Indian Forester 76: 244-253.

Bakshi BK. 1958. New records of hymenomycetes in India. Indian Phytopathology. 11: 88.

Bakshi BK. 1971. The *Polyporaceae* (On Trees and Timber). ICAR, New Delhi 58-62, 246.

Bondarzew AS. 1953. The Polyporaceae of the European USSR and Caucasia. U.S.S.R Academy of Sciences Printing House, Moscow. [in Russian].

Corner EJH. 1991. Ad polyporaceas. VII. Beih. Nova Hedw. 101: 1-175.

Cunningham GH. 1965. *Polyporaceae* of New Zeland. New Zealand Department Scientific and Industrial Research Bulletin. 164: 1-304.

Dai YC. 1999. *Phellinus* sensu lato (*Aphyllophorales. Hymenochaetaceae*) in East Asia. Acta Bot. Fenn. 166: 1-115.

Dai YC, Xu MA. 1998. Studies on the medicinal polypore, *Phellinus baumii*, and its kin *P. linteus*. Mycotaxon 67: 191-200.

Domanski S, Orlos H, Skirgiello A. 1973. Fungi. *Polyporaceae* II (pileate), *Mucronoporaceae* II (pileate), *Ganodermataceae*, *Bonderzewiaceae*, *Boletopsidaceae*, *Fistulinaceae*. Foreign Scientific Publications, Poland.

Donk MA. 1974. Checklist of European polypores. Royal Netherlands Academy of Arts and Science Press, Netherlands.

Fiasson JL. 1982. Distribution of styrylpyrones in the basidiocarp of various *Hymenochaetaceae*. Bioch. Syst. Ecol. 10: 289-296.

Fiasson JL, Niemelä T. 1984. The *Hymenochaetales*: a revision of the European poroid taxa. Karstenia 24: 14-28.

Fidalgo O. 1968. *Phellinus* from Venezuela – I. Mem. New York Bot. Gard. 17: 109-147.

Fischer M. 1987. Biosystematische Untersuchungen an den Porlingsgattungen *Phellinus* Quél. und *Inonotus* Karst. Bibl. Mycol. 107: 1-133.

Fischer M. 1996a. Molecular and microscopical studies in the *Phellinus pini* group. Mycologia 88: 230-238.

Fischer M. 1996b. On the species complexes within *Phellinus*: *Fomitiporia* revised. Mycol. Res. 100: 1459-1467.

Ganesh PN, Leelavathy KM. 1986. New records of *Phellinus* from India. Curr. Sci. 55: 727-728.

Gilbertson RL. 1979. Wood rotting fungi of North America. Mycologia 72: 1-49.

Gilbertson RL, Ryvarden L. 1986. North American Polypores. Vol.1, Fungiflora, Norway.

Gilbertson RL, Ryvarden L. 1987. North American Polypores. Vol. 2, Fungiflora, Norway.

Goés-Neto A, Loguercio-Leite C, Guerrero RT. 2001. Morphological cladistic analysis of tropical *Hymenochaetales* (Basidomycota). Mycotaxon 79: 467-479.

Hansen L, Knudsen H. 1997. Nordic macromycetes. Vol. 3. Helsinki University Printing House, Finland.

Jahn H. 1981. Die resupinaten *Phellinus* – Arten in Mitteleuropa. Nachträge 1967-1981. Bibl. Mycol. 81: 109-151.

Jordan M, David C. 1995. Encyclopedia of Fungi of Britain and Europe. David and Charles Book, UK.

Jülich W. 1984. Basidiomycetes of South-East Asia. Persoonia 12: 107-117.

Kim S-Y, Jung HS. 2002. Cladistic analysis of the Polyporaceae using morphological characters. Mycotaxon 82: 295-314.

Kotlaba F, Pouzar Z. 1978. Revision of the original material of *Phellinus sulphurascens* Pil., *Xanthochrous glomeratus* ssp. *heinrichii* and *Polyporus rheades* Pers. (Hymenochaetaceae). Česká Mycologie 24: 146-152.

Larsen MJ, Cobb-Poulle LA. 1990. *Phellinus* (*Hymenochaetaceae*). A survey of the world taxa. Synopsis Fungorum 3: 1-206.

Lim YM, Lee JS, Jung HS. 2003. Type studies on *Phellinus baumii* and *Phellinus linteus*. Mycotaxon 85: 201-210.

Lloyd CG. 1915. Synopsis of the genus *Fomes*. Mycological Writings 4: 209-288.

Lowe JL. 1957. Polyporaceae of North America. The genus *Fomes*. State University College of Forestry at Syracuse University Technical Publication 80: 1-97.

Mizuno T. 2000. Development of an antitumour biological response modifier from *Phellinus linteus* (Berk. et Curt.) Teng (Aphyllophoromycetideae). Int. J. Med. Mushr. 2: 21-33.

Natarajan K, Kolandavelu K. 1985. Resupinate Aphyllophorales from South India – I. Kavaka 12: 71-76.

Niemelä T. 1972. On Fennoscandian Polypores. II. *Phellinus laevigatus* (Fr.) Bourd. & Galz. and *P. lundellii* Niemela, n. sp. Ann. Bot. Fenn. 9: 41-59.

Niemelä T. 1975. On Fennoscandian polypores 4. *Phellinus igniarius*, *P. nigricans* and *P. populicola*, n. sp. Ann. Bot. Fenn. 12: 93-122.

Nuss I. 1986. Zur Ōkologie der Porlinge II. Bibl. Mycol. 105: 1-299.

Omar MB, Bolland L, Heather WA. 1979. A permanent mounting medium for fungi. Bull. Br. Mycol. Soc. 13: 31-32.

Overholts LO. 1929. Research methods in the taxonomy of Hymenomycetes. Proceedings of the International Conference of Plant Sciences 2: 1688-1712.

Overholts LO. 1953. The *Polyporaceae* of United States, Alaska and Canada. University of Michigan Press, U.S.A.

Owens CF. 1936. Studies on the wood rotting fungus *Fomes pini*. Variation in morphology and growth habit. Am. J. Bot. 23: 144-149.

Parmasto E. 1985. Species concept in *Hymenochaetaceae* (Fungi, *Hymenochaetales*). Proc. Ind. Acad. Sci. 94: 369-380.

Parmasto E. 1988. What is *Ochroporus ossatus* (*Hymenochaetaceae*)? Mycotaxon 32: 219-222.

Rabba AS. 1994. Studies in the genus *Phellinus* Quél. from Maharashtra. Ph.D. thesis. University of Pune, India.

Rabba AS, Vaidya JG, Nanda MK. 1994. The genus *Phellinus* from Bhimashankar forests. Biol. Ind. 5: 47-56.

Rajchenberg M. 1989. *Polyporaceae* (*Aphyllophorales*, Basidiomycetes) from southern South America: A mycogeographical view. Sydowia 41: 277-291.

Roy A. 1979. Taxonomy of *Fomes durissimus*. Mycologia 71: 1005-1009.

Ryvarden L. 1972. A critical checklist of the *Polyporaceae* in tropical East Africa. Norw. J. Bot. 19: 229-238.

Ryvarden L. 1978. *Polyporaceae* of North Europe. Fungiflora, Norway.

Ryvarden L. 1987. New and noteworthy polypores from tropical America. Mycotaxon 28: 525-541.

Ryvarden L. 1991. Genera of polypores, nomenclature and taxonomy. Synopsis Fungorum 5: 1-363.

Ryvarden L, Gilbertson RL. 1994. European polypores part 2. Synopsis Fungorum 7: 394-743.

Ryvarden L, Johansen I. 1980. A preliminary polypore flora of East Africa. Fungiflora, Norway.

Sharma JR. 1993. New records of polypores from India. Ind. J. For. 16: 186-187.

Singh B. 1966. Timber decay due to five species of *Fomes* as new record in India. Indian Forester 92: 653-655.

Smith JE, Rowan NJ, Sullivan R. 2002. Medicinal Mushrooms: their therapeutic properties and current medicinal usage with special emphasis on cancer treatments. Cancer Research, UK (URL http://sci.cancerresearchuk.org/labs/med_mush/med_mush.html).

Swofford DL. 1999. PAUP* 4.0: phylogenetic analysis using parsimony, version 4.0b10. Sinauer Associates Inc., U.S.A.

Thind KS, Chatrath MS. 1957. The *Polyporaceae* of the Mussoorie Hills II. Research Bulletin of the Punjab University 125: 431-442.

Thind KS, Dhanda RS. 1980. The *Polyporaceae* of India. X. Kavaka 8: 59-67.

Tiwari DP, Harsh NSK, Tiwari CK. 1989. Polypore flora of Jabalpur and its eastern Suburbs. Biomes 4: 69-79.

Vaidya JG. 1995. Biology of the Fungi. Satyajeet Prakashan, India.

Vaidya JG, Bhor GL. 1991. Medicinal important wood rotting fungi with special emphasis on phansomba. Deerghayu International 7: 16-19.

Vaidya JG, Lamrood PY. 2000. Traditional medicinal mushrooms and fungi of India. International Journal of Medicinal Mushrooms 2: 209-214.

Vaidya JG, Rabba AS. 1993. Fungi in folk medicine. Mycologist 7: 131-133.

Wagner T, Fischer M. 2002. Proceedings towards a natural classification of the worldwide taxa *Phellinus* s.l. and *Inonotus* s.l., and phylogenetic relationships of allied genera. Mycologia 94: 998-1016.

MYCOTAXON

Volume 95, pp. 133–136 *January–March 2006*

Paecilomyces verticillatus, a new species isolated from soil in China

Zhu Li, Yanfeng Han
& Zongqi Liang*

zhuliluck@ 163.com swallow112886@yahoo.com.cn
**zqliang472@yahoo.com.cn*

Institute of Fungus Resources, Guizhou University
Guiyang 550025, China

Abstract—A new species, *Paecilomyces verticillatus*, isolated from soil samples of Guiyang City, Guizhou Province, China, is described and illustrated. It can be distinguished by white colonies when grown on Czapek agar, whorls of phialides arising from undifferentiated hyphae, and subglobose conidia.

Key words—fungi, hyphomycete, taxonomy, morphology

Introduction

Samson (1974) discussed the morphology differences between *Verticillium* Nees and *Paecilomyces* Bainier. The genus *Verticillium* is characterized by verticillate conidiophores, awl-shaped phialides and conidia in slimy heads. However, *Verticillium* sect. *Prostrata* contains some species with *Paecilomyces*-like, slightly swollen phialides and conidia in chains. Our new species, *Paecilomyces verticillatus*, resembles *Verticillium* species that produce phialides always in whorls on aerial hyphae or simple conidiophores. The fact that the phialides in our proposed new species typically have inflated bases with abruptly tapering necks places it in the genus *Paecilomyces*.

Materials and Methods

Collection and strains isolation

Strain GZDX-IFR49-01 was isolated from soil collected from Forest Park, Guiyang, Guizhou Province, China, May, 2003. Two grams of soil were added to a flask containing 20ml sterilized water and glass beads. The soil suspension was diluted to a concentration of 10^{-1}-10^{-3} after shaking for about 10min. A 1ml soil suspension (concentration 10^{-3}) was mixed with Martin medium in sterilized 9 cm diam Petri dish and incubated at 25°C for 5 days. Colonies showing a typical *Paecilomyces* conidiogenous structure were then purified by transplanting to Martin's slants.

---

*Corresponding author: Zongqi Liang

Identification of strains

The strains were transplanted onto Czapek agar, potato dextrose agar (PDA), and Sabouraud agar in accordance with procedures set forth in Brown and Smith (1957) and Samson (1974). After incubation at 25-26°C for 14 days, strains were identified based on colony characters, conidiogenous structures, and other biological features.

The type culture GZDX-IFR49-01 and the holotype GZDX49-01 of *P. verticillatus* (a dried plate culture on Czapek agar) were deposited in the Institute of Fungus Resources, Guizhou University, China.

Description of new species

Paecilomyces verticillatus Z.Q. Liang, Z. Li & Y.F. Han **sp. nov.** **Figs. 1-6**

> *On agaro Czapekii coloniae 35-55 mm diam in 14 diebus ad 25°C, albae, floccosa, margine irregularis, reserso albae. Hyphae hyaline, levia, 1.2-1.8 µm crasssa. Conidiophora breva, 9.6-19.8 µm longa. Phialides 7.8-14.4 × 1.2-2.4 µm, verticillatis, conis vel bases cylindres. Conidia hyalina, levia, subglobasa vel ellipsoidea, catenulatae, 1.2-1.8 × 0.6-1.2 µm.*
>
> *Typus: GZDX-IFR49-01 et cultara viva GZDX49-01, isolatus ab soli in sylvis, Guiyang, Provinice Guizhou, V, 2003, Y. F. Han & H. L. Chu; in Guizhou Univ., conservatur.*

Colonies on Czapek agar growing rapidly, attaining a diameter of 35-55 mm within 14 days at 25°C; ridgy, white, floss, irregular in the margin; reverse white. Vegetative hyphae septate, hyaline, smooth-walled, 1.2-1.8 µm wide. Conidiophores hyaline, smooth-walled, 9.6-19.8 µm, directly bearing a whorl of 3 to 5 phialides or verticillate prophialides with a whorl of 3 phialides. Phialides 7.8-14.4 × 1.2-2.4 µm, awl-shaped or consisting of a cylindrical basal portion and a thin neck, less than 0.5 µm wide. Conidia one-celled, hyaline, smooth-walled, mostly subglobose to ellipsoidal, 1.2-1.8 × 0.6-1.2 µm; few fusiform, 1.8-3.0 × 1.8-2.4 µm, forming divergent, dry chains or aggregating spore group.

Distribution: Guizhou Province, China.

Discussion

Seven accepted species of *Paecilomyces* with subglobose conidia were identified using CABI Bioscience Database, CBS Database and recent papers (Table 1).

Table 1. Seven accepted species of *Paecilomyces* with subglobose conidia

P. militaris Z.Q. Liang
P. niveus Stolk & Samson
P. odonatae Zuo Y. Liu et al
P. rariramus Z.Q. Liang & B. Wang
P. stipitatus Y.F. Han & Z.Q. Liang
P. vinaceus Y.F. Han & Z.Q. Liang
P. viridis Segretain et al. ex Samson

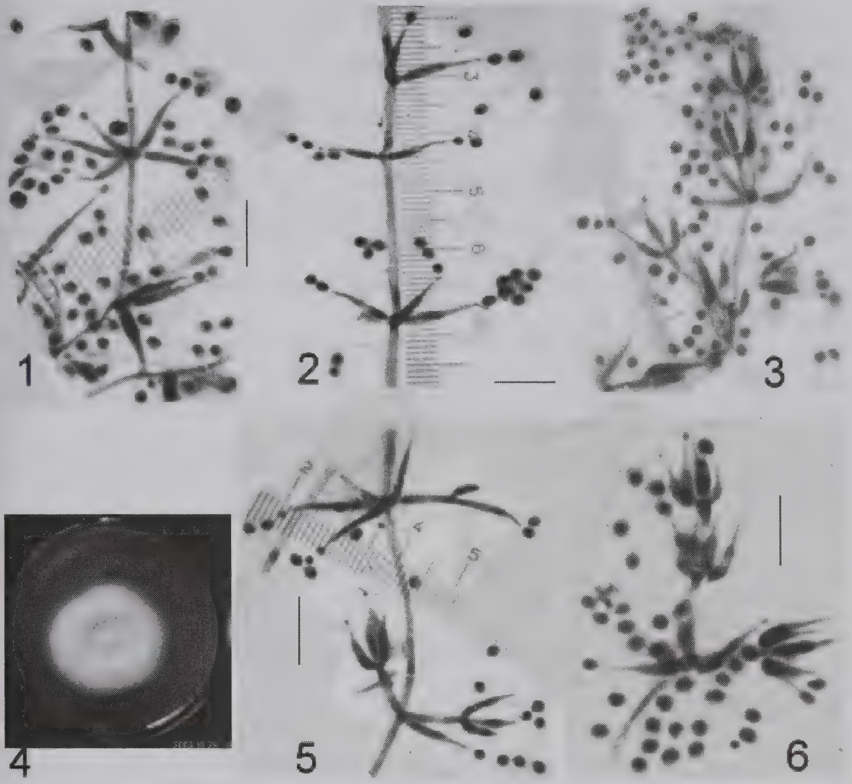


Fig. 1-6 Colony and conidiogenous structure of *Paecilomyces verticillatus*
1-3, 5-6. Conidiogenous structure. 4. Colony Bar = 10 μm.

The thermophilic nature of *P. niveus* (Samson 1974), the yellow-green colony reverse of *P. viridis* (Samson 1974), the vinaceous colony reverse of *P. vinaceus* (Han et al. 2005), the rarely branching conidiogenous structures of *P. rariramus* (Liang 2003), and the larger conidia (1.7-5.9 × 1.7-3.3 μm) and yellow colony reverse of *P. stipitatus* (Han et al. 2005) do not exist in *P. verticillatus*.

P. odonatae (Liu et al. 1996) and *P. militaris* (Liang 2001) are closely related to the new species. However, *P. odonatae* has both globose conidia in straight chains and cylindrical conidia in oblique chains. *P. militaris* also exhibits both straight and oblique chain conidial arrangements and produces larger conidia (2-3.2 × 1.5-2 μm) than those of *P. verticillatus*.

Paecilomyces verticillatus can be distinguished from all the other species in the genus by producing (1) phialides that often arise from undifferentiated hyphae in whorls, (2) white colonies on Czapek agar, and (3) subglobose conidia.

Acknowledgements

This project was supported by the National Natural Science Foundation of China (No. 30270011). We are grateful to Professors Zuoyi Liu and Jichuan Kang for their comments on the manuscript. At the same time, we sincerely appreciate editorial review and revisions.

References

Brown AHS, Smith G. 1957. The genus *Paecilomyces* Bainier and its perfect stage *Byssochlamys* Westling. Trans. Brit. Mycol. Sac. 40 (1): 17 - 89.

Han YF, Chu HL, Liang ZQ. 2005. Two new species of the genus *Paecilomyces* in China. Mycotaxon 92: 311 - 316.

Liang ZQ, Wang B, Kang JC. 2003. Several rare entopathogenic fungi from western Sichuan Mountains. Fangal Diversity 12: 129 - 134.

Liang ZQ. 2001. A corroboration of the anamorph of *Cordyceps militaris – Paecilomyces militaris* Liang sp.nov.. Acta Edulis Fungi 8 (4): 28 - 32.

Liu ZY, Liang ZQ, Liu AY. 1996. A new species *Paecilomyces* isolated from *Cordyceps odonatae*. Mycosystema 9: 83 - 87.

Samson RA. 1974. *Paecilomyces* and some allied Hyphomycetes. Stud. Mycol. 6: 1 – 119.

MYCOTAXON

Volume 95, pp. 137–180 January–March 2006

A monograph of the genus *Cookeina* (Ascomycota, *Pezizales, Sarcoscyphaceae*)

Teresa Iturriaga & Donald H. Pfister

titurri@usb.ve
Departmento de Biología de Organismos, Universidad Simón Bolívar
Caracas, Venezuela

dpfister@oeb,harvard.edu
Department of Organismic and Evolutionary Biology, Harvard University
Cambridge, MA, USA

Abstract—Eight species of the wood inhabiting pantropical genus *Cookeina* are described and illustrated. The genus *Cookeina* is characterized by large, stipitate or sessile brightly colored apothecial ascoma, with or without hairs, and by distinctive, thick-walled asci that have eccentricly placed opercula. An overview of the morphology, development and life histories of the species are given along with discussion of their relationships. A new species, *C. colensoiopsis*, is described from Venezuela, *C. speciosa* is recognized as a species complex, and a lectotype is designated for *C. sinensis*.

Key words—Pezizomycetes, cup-fungi, taxonomy

Table of Contents

Introduction 138
Historical background 138
Materials and methods 139
Morphology and distribution 140
  Macroscopic features 140
  Asci 142
  Ascospores 142
  Paraphyses 143
  Hairs and outer surfaces 143
  Excipular construction 143
  Development 144
  Spore discharge 144
  Anamorphic state 145
  Distribution 145
  Phylogeny 146
Taxonomic treatment 146
  Key to species 147
  Descriptions 147
    Cookeina colensoi 147
    Cookeina colensoiopsis 151
    Cookeina indica 154
    Cookeina insititia 155
    Cookeina sinensis 159
    Cookeina speciosa 160
    Cookeina tricholoma 165
    Cookeina venezuelae 170
  Excluded species 174
Acknowledgements 176
Literature cited 176

Introduction

In this monograph we present an account of the genus *Cookeina*, a member of the *Pezizales,* which is comprised of species with lowland tropical and subtropical distributions. Species in the genus inhabit recently fallen angiospermous woody substrates and are presumed to be saprotrophic. The apothecial ascomata are brightly colored, range in size up to about 3 cm in diameter and are often stipitate. Because they are large, brightly colored and sometimes occurr in profusion in a single small area, members of this genus are frequently collected. Even so, distributional records are incomplete and primarily account for the common species. Nonetheless, the knowledge of the biology of the species is minimal.

The genus has been placed in the *Sarcoscyphaceae* by all modern authors (Denison 1967, Eckblad 1968, Korf 1970, 1972, 1973, Le Gal 1953, Rifai 1968) and it has been well characterized based on macro- and microscopic features. Circumscription has been debated only in regard to the placement of *Peziza insititia*. This taxon has been placed in *Cookeina* or alternatively in the monotypic genus *Boedijnopeziza* (Ito & Imai 1937). Recently, Melendez-Howell et al. (2003) and Weinstein et al. (2002) include *Boedijnopeziza* in *Cookeina*. Within the *Sarcoscyphaceae Cookeina* has been allied most closely to *Microstoma*. Together *Cookeina* and *Microstoma* form a monophyletic group that has been recognized formally as the tribe *Boedijnopezizeae* (Cabrello 1988, Harrington et al. 1999, Korf 1970, 1972, 1973).

In this work we summarize current knowledge about the genus and provide identification keys, descriptions, and commentaries. We bring together in a single source the literature related to this genus. We are aware of certain gaps related to distributional records, particularly regarding Africa.

Historical background

Dating from the earliest days of biodiversity exploration in the Caribbean, the history of the genus *Cookeina* spans three hundred years. In 1705 an illustration of *Peziza speciosa* from the West Indies was published (Plumier 1705). The figure caption in Latin and French, a polynomial and the only description we have of Plumier's collections, reads *"Fungoides cyathiforme coccineum, oris pilosis/Foungoide en maniére de verre couleur d'écarlete, à bord velu."* With this brief commentary, the defining macroscopic characters of this fungus and of the genus *Cookeina*, to which it would ultimately be referred, were set down. These fungi are goblet-shaped, scarlet to carmine, with hairs at the mouth or margin of the apothecial ascomata. Although recorded by Fries (1822) under *Peziza speciosa* this early record went largely unnoticed until Dennis (1994) provided an intrepretation of Plumier's illustration that placed it in the genus *Cookeina*. Under the broad, inclusive genus *Peziza* other species sharing these characteristics were described in the years following Fries. As tropical areas of the world were explored during the middle years of the 19th century several species were added, which ultimately were treated in the genus *Cookeina*. The short descriptions from this period often lacked essential details of spore size and hair morphology resulting in the production of many synonymous names for the common species. In the late 19th century, as part of a trend toward erecting segregate genera for the larger members of the *Pezizales,* several authors nearly simultaneously created genera to accommodate these distinctive species. Cooke (1879) placed *P. sulcipes*, *P. hindsii*, *P. tricholoma* and *P. insititia*, all referable to *Cookeina* today, in *Peziza* subgenus *Trichoscypha*. Saccardo (1889) raised Cooke's subgenus *Trichoscypha* to generic level but in doing so created a later homonym. *Trichoscypha* Hooker f. (Bentham & Hooker 1862) is a genus in the *Anacardiaceae*. Further confusion exists because Boudier (1885) used *Trichoscypha* as a generic name for inoperculate discomycetes mostly belonging to the genus *Lachnellula* (*Hyaloscyphaceae*). Saccardo (1889) included species with both operculate and inoperculate asci in *Trichoscypha*. When Kuntze (1891) erected the genus *Cookeina* his circumscription created a highly heterogeneous assemblage of taxa including members of both the *Pezizales* and *Helotiales* but encompassed those taxa previously treated in *Trichoscypha*. Finally, *Pilocratera* was described (Hennings (1891). It post-dates the creation of *Cookeina* but was in general use for a period. Seaver (1927) designated *P. tricholoma* as the type species of the genus *Cookeina;* all authors have followed this typification. *Cookeina, Trichoscypha* and *Pilocratera*, are all based on the same type species (Eckblad, 1968).

Seaver (1913, 1925, 1927, 1928, 1936, 1942) studied the American species and codified both the use of the name and the concept of the genus *Cookeina*. At the same time, Boedijn (1929, 1933) studied and described Asian materials. Boedijn's observations on spore germination and ascus construction contributed to the overall knowledge of the genus. With some hesitation Seaver (1925) added *C. tetraspora* to the genus. This species differs in construction of the excipulum and in spore morphology. Later, it was designated the type species of the genus *Nanoscypha* (Denison 1972). Le Gal (1953) regularized the taxonomy through study of type material and elucidated the ascus morphology, as discussed more fully below. Weinstein et al. (2002) discussed relationships of the species based on a molecular phylogenetic study. Several authors treated one or more species from specific geographical regions as follows: **Africa and Madagascar** (Alasoadudra 1972, Douanla-Meli & Langer 2005, Le Gal 1953, 1960, Moravec, 1997), Indonesia (Boedijn 1929,1933), the **Americas** (Calonge 1986, Chacón & Medel 1990, Denison 1963, 1967, Dennis 1970, Gamundí, 1957, 1959, 1971, Hanlin et al. 1991, Hanlin 1993, Romero & Gamundí 1986, Tobon 1991), the **West Indies** (Dennis 1954, Maldonado González 2000, Patouillard in Duss 1903, Pfister 1974, Seaver 1936, Vooren 2002, 2003), Asia and India (Durrieu et al. 1997, Liou & Chen 1977, Nagao 1997, Otani 1971, 1975, Pfister & Kausal 1984, Wang 1997, Wang 2001, Yang 1990) and **Australasia** (Rifai 1968).

Materials and methods

This study used herbarium specimens and living material, particularly from South America. Type specimens of all taxa were examined when available. A concerted effort was made to collect and document specimens during two summers in an Amazonian forest in the southern part of Venezuela. In that study color variation in *Cookeina speciosa* was a particular focus. Collections from that study were deposited in the following herbaria: FH, USB, VEN.

Dried specimens were prepared for microscopic examination by rehydrating a small portion of an apothecium in water for 1-2 hours. The sample was then oriented on the stage of a sliding freezing microtome, covered with 50% aqueous commercial mucilage and frozen. Median sections 20 to 50 µm thick were made. Sections were removed from the blade with a small, wet brush and placed on a microscope slide, where they were either mounted directly for examination or left to air-dry on the slide. In either case sections were selected under the dissecting scope, transferred with an insect pin to a slide and mounted in water, Cotton Blue in lactic acid or Congo Red in ammonia. Where quality and condition allowed 10 to 30 measurements of each structure were recorded for each set of sections. Only asci that contained mature ascospores were measured. Ascus measurements do not include the thin hyphal base. Ascospore sizes are based on the measurement of discharged ascospores; colors are given for fresh, dry and re-hydrated material. Drawings were made using a measured free hand method.

Terminology for cell types and arrangements follows Korf (1952, 1973) and Pfister & Kimbrough (2001). Herbarium acronyms follow *Index Herbariorum* (www.nybg.org/bsci/ih/ih.html); author's names are abbreviated following *Authors of Fungal Names* (www.indexfungorum.org/AuthorsOfFungalNames.htm).

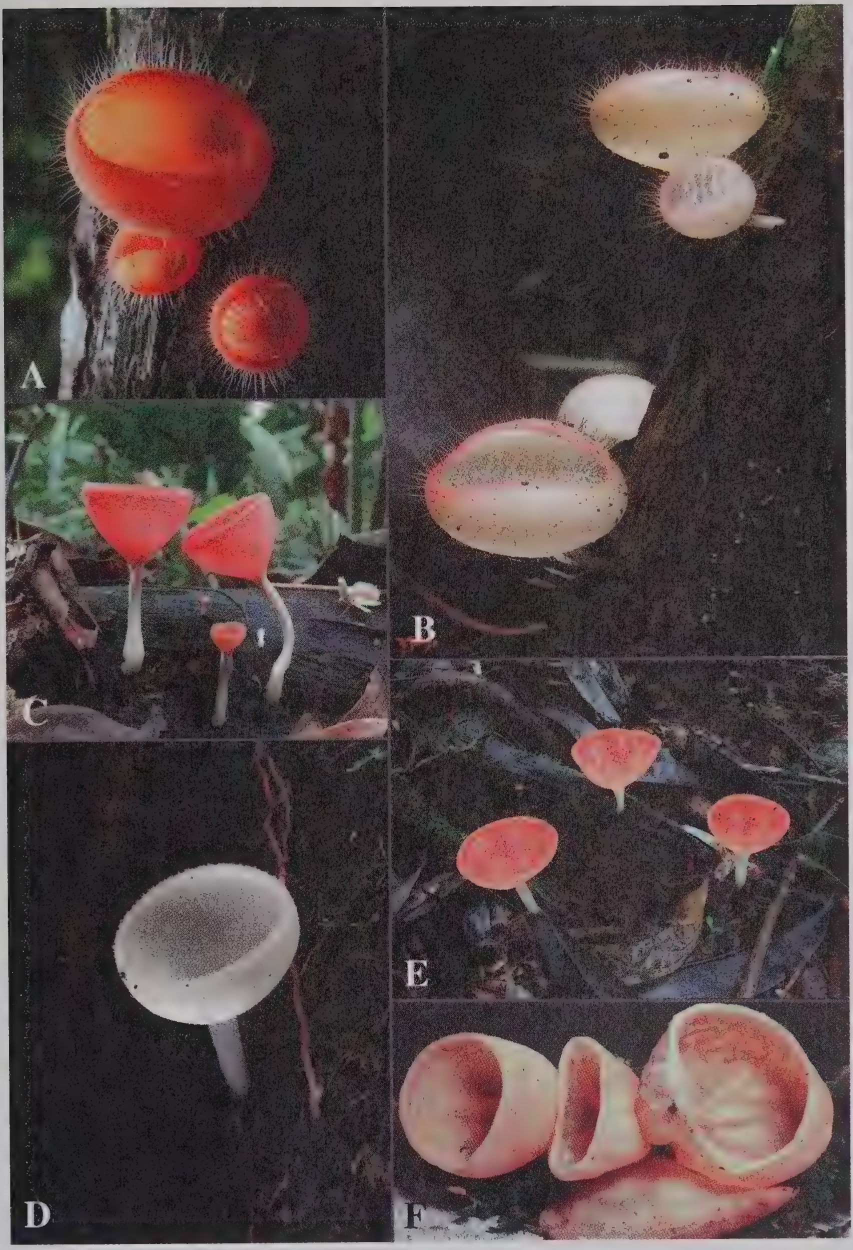
In the nomenclators the notation "!!" following citations indicates, that holotype, neotype, or isotype material was examined. The notation "!" indicates that syntype, paratype, or other authentic material was examined. A question mark before a species name indicates that type material was not examined but that the indicated placement is probable. Specimens examined are cited with the data as they appear on the packet label, supplemental data are enclosed in square brackets. Other abbreviations used are HT = holotype, IT = isotype, PT = paratype, and NT = neotype. Over the course of these studies many collections were consulted most are listed in the specimen cited section but citations have been shortened and a few collections that were consulted incidentally have not been listed.

Results and general discussion

Macroscopic features

Apothecia of *Cookeina* species are relatively large, ranging up to 3 cm in diam. They are characteristically pliable, resilient and not easily broken by handling. Carotenoids are present (Arpin 1969) which give the hymenia colors ranging from yellow and orange to scarlet and dark tones of mauve (see figure 1). In a few cases ascomata are white; in others maroon to brown. Apothecia of *C. colensoi* and *C. venezuelae* have short stipes or are nearly sessile. When present, stipes may range in length up to 8 cm. The outer surface of the ascomata, or the receptacle, may have distinct hairs distributed either over the entire surface, as in *C. tricholoma* and *C. sinensis*, or at the margins, as in *C. insititia* and *C. speciosa*. *Cookeina colensoi, C. indica* and *C. venezuelae* lack prominent hairs. Hairs are composed of fascicles of fused hyphae originating from the ectal or the medullary excipulum. Receptacle surfaces of all species are to some degree tomentose due to the projecting free hyphae that arise from the ectal excipulum.

Figure 1. Habitat photographs of species of *Cookeina*. A. *C. tricholoma* TL-11427 (C). B. *C. tricholoma* TL 11409 (C). C. *C. speciosa* TL 8405 (C). D. *C. speciosa* TL – 11393 (C). E. *C. speciosa* TL- 11475 (C). F. *C. venezuelae* Halling 5452 (NY). A-E courtesy of Jens Petersen (copyright Jens H. Petersen/MycoKey) specimens from Ecuador, and F. was used with the permission of Roy Halling, specimen from Venezuela.


A
B
C
D
E
F

Asci

Asci are large, up to 550 µm long and 40 µm broad and have thick walls. No part of the wall colors blue in iodine solutions; wall layers are differentially stained in Congo red in ammonia and the operculum stains more deeply in Congo red than surrounding regions of the wall. The long cylindrical asci are rounded proximally and distally. Asci have prominent, apically thickened opercula eccentrically located at the ascus apex. The asci open along a line of dehiscence to form the operculum. The thickened operculum is hinged at the lower region and it folds back at the time of rupture. Samuelson (1975) described the apical apparatus using TEM and Melendez-Howell et al. (2003) gave detailed information on ascus wall-layering using TEM. The asci are produced from narrow ascogenous hyphae which expand abruptly at the base of the asci. This gives the asci a rounded base. No croziers were observed. Because of the thinness of the hyphae at the ascus base, the hymenium often separates from the subhymenium when preparing mounts for microscopy. In all species asci mature synchronously. Within a single ascoma all ascospores are at exactly the same stage of development. The controls involved in this process are unknown. Asci that are constricted basally and that mature simultaneously are known in *Cookeina*, its sister genus *Microstoma* and in *Chorioactis* Kupfer ex Eckblad (Pfister & Kurogi 2004) which is placed in the *Sarcosomataceae.*

Le Gal (1953), Eckblad (1968) and Samuelson (1975) discussed the construction of the ascus in *Cookeina* species with a particular emphasis on their apical structure. The eccentric position and thickening of the operculum have been variously interpreted. The notation of such asci as "suboperculate" has been discussed by these authors.

Ascospores

Ascospores are pink or buff in deposit and range up to 52 µm long and 21 µm wide. They are ellipsoid, fusoid, or naviculate often appearing flattened on one side or curved, as in *C. insititia*. In light microscopy ascospores are smooth or have low longitudinal ridges or grooves and occasionally have transverse folds. The ridges may be unbroken from pole to pole or they may form disjunct rows that may anastomose. Berkeley (1875), who was the first to observe the markings, mentioned that one specimen of *C. tricholoma* had "longitudinal dots as a Diatom." SEM studies of the smooth-spored species show some divergence. Melendez-Howell et al. (2003) illustrated low ridges and reticulations on the surface of ascospores of *C. insititia* and Moravec (1997) depicted similar markings in *C. colensoi* but Weinstein et al. (2002) indicate that the spores are smooth.

In some species the ascospore walls are thickened at the poles, forming short apiculae. Apiculae are well developed in *C. speciosa* and *C. venezuelae*, whereas in *C. tricholoma* the walls are thickened but the spores are not conspicuously apiculate. In other species polar thickenings are variably present. Boedijn (1933) found that in these species germ tubes originated from the apiculae. He observed that *C. insititia* has a single wall layer; Pfister (1973) verified this and noted that the other species of *Cookeina*, including *C. colensoi* and *C. venezuelae*, all have double spore walls. Pfister (1973) stated that these walls separate readily in 10-15% KOH. Melendez-Howell et al. (2003) through TEM studies showed that the perispore layer of *C. insititia* is very thin and that the spores have one wall layer or "paroi proper" as compared to the multiple layers present in *C. tricholoma*. TEM studies of *C. speciosa* (as *C. sulcipes*) failed to show clear ascospore wall layers (Melendez-Howell et al. 2003).

Berthet (1964) reported, and Pfister (1978a) confirmed, that ascospores are multinucleate, a character of the family *Sarcoscyphaceae*. Ascospores always contain oil guttules. There may be a single guttule, several large guttules or numerous small droplets; guttulation is somewhat variable within a species. There is a tendency for the guttules to fuse and amalgamate in drying and rehydration and, although the presence or absence of guttules is an important character, their number and arrangement is not reliable, particularly in dried specimens.

Paraphyses

Paraphyses are 1-6 µm wide at the apex, hyphoid and are sometimes slightly thinner below the apex but are not prominently enlarged above. Their cells form lateral projections that frequently fuse with cells of adjacent paraphyses. The result is a tight, interwoven, three-dimensional network surrounding the asci, among which individual paraphyses are often difficult to discern. For this reason some authors have said that paraphyses are lacking. We have seen paraphyses in all material examined. The apices are free and in some collections extend above the hymenium. In *C. colensoiopsis* and *C. speciosa* the terminal cells form setae that project above the level of the hymenium. Berthet (1964) reports the cells of the paraphyses to be multinucleate.

Hairs and receptacle surface

In all taxa, the surface of the receptacle is minutely tomentose or granulose due to the presence of globose to angular cells on the outer surface. These cells give the receptacle a pruinose appearance. In addition, short projecting hyphae, only clearly seen under the microscope, produce a tomentum. The tomentum may show two morphologies: 1) individual monilioid processes that arise from the margin and the receptacle. These elements are composed of 2–5 short hyaline cells with thick and rugose walls; 2) fused triangular bundles of cells at the margin that are wide at the base and narrow towards the apex.

True hairs are easily seen with the unaided eye and may reach a length of 7 mm. They are composed of adherent hyphal filaments running parallel to one another. The hairs arise from the cells of the outer excipulum and are located in 3-5 rows at the margin of the disc except in *C. sinensis* and *C. tricholoma* where the hairs arise from the medullary excipulum and are more or less evenly distributed over the outside of the receptacle and the stalk (fig. 2).

Excipular construction

The patterns of excipular organization vary only in minor detail from species to species. The ectal excipulum is composed of two layers: an outer ectal excipulum up to 175 µm thick, of *textura globosa* to *textura angularis*, the cells of which are arranged in indistinct rows of 3 to 7 cells. These cells have thick walls, especially the cells toward the outer surface where they also become rounded. The inner ectal excipulum is a layer at the junction between the medullary and outer ectal excipulum. It is composed of *textura intricata* or *textura oblita*, of loosely interwoven, septate, branched, thin–walled hyphae. In some cases the hyphae are immersed in a distinct gelatinous matrix with hyphae oriented perpendicular to the surface of the receptacle. This gelatinous layer can be seen as a light refractive continuous band in median sections. A gel layer is present in *C. colensoi*, *C. colensoiopsis*, *C. insititia* and *C. venezuelae*. Despite its prominence gel has often been overlooked in these species. We occasionally saw gel in other species

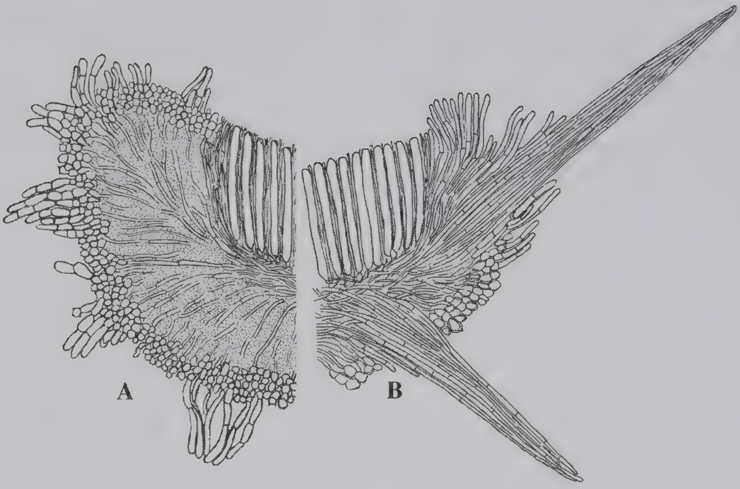


Figure 2. *Cookeina venezuelae* and *C. tricholoma*, cross sections of apothecia showing tissues and hairs. A. *C. venezuelae*, stipled regions indicate gelatinous tissues, hairs are superficial and arise from the ectal excipulum. B. *C. tricholoma*, no gelatinous tissues are present and hairs arise from the medullary excipulum.

but in those cases it occurred in isolated pockets within the excipulum rather than as a continuous, uniform layer and was variable within even a single collection.

The medullary excipulum, the innermost layer, which forms the core of the apothecium, is composed of *textura porrecta* to *textura intricata*. It is built up of narrow, septate, somewhat thick-walled hyphae that run parallel to the outer surface of the apothecium. The subhymenium, the layer beneath the hymenium, from which asci arise, is of dense *textura intricata*.

Development

Only *C. tricholoma* has been studied in any detail regarding apothecial development (Pfister 1978a). Based on a study of primordia collected in the field the hymenium is exposed in the youngest material observed. Pfister (1978a) suggested that all *Sarcoscyphaceae* develop in agymnohymenial fashion; this has yet to be challenged or confirmed across the entire family. Boedijn (1933) reported that in juvenile states the hymenium of *C. insititia* is covered by a "nearly continuous subconical sheath, which by expansion of the cup ruptures radially and gives rise to a ring of hairs." Wang (1997) made similar observations. We have made no attempt to comparatively study primordial states in this work.

Spore discharge

In all species asci mature simultaneously. Boedijn (1933) observed that ascospores were discharged mostly in an oblique direction due to the eccentricity of the ascus opening. Both Seaver (1928) and Boedjin (1933) commented on ascospore discharge in *Cookeina*, most probably in *C. speciosa*, based on their drawings of the ascospores.

Boedjin claimed that the ascus jet (ascospores and epiplasm) passed through the ascostome (ascus pore) all at once, while Seaver (1928) suggested there was a contraction between each individual spore ejection. In clarifying his earlier observation Seaver (1942) said that he had not stated there was a pause between each ascospore being ejected, as implied by Boedijn (1933), but that there was an expansion and contraction of the ascostome between each spore ejection.

Despite the uniform maturation of the asci, Boedijn (1933) noted that ascospores were discharged over a period of several days. Zoberi (1973) showed that as water evaporated from the apothecium, or when it was removed mechanically, ascospore discharge occurred. All spores were discharged within a 5-hour period.

One of our observations from the field relates to Zoberi's experimental work. In the stipitate taxa the cup may fill with water during rains. As these cups fill the added mass causes the tough and resilient stipes to bend and dump out the accumulated water. Because the stipes are resilient and flexibile the cup resumes its previous position and is undamaged. Thus, the cups can fill, be emptied and be hydrated for ascospore discharge.

Anamorphic state, growth and cultivation

Boedijn (1929, 1933) reported germination of ascospores of *Cookeina tricholoma* and *C. speciosa* as *C. sulcipes*. He noted three types of germination. One in which one or two germ tubes were formed but no conidia, a second in which spores produced numerous short germ tubes, each of which bore several conidia, and a third type in which several germ tubes formed, elongated but only rarely produced conidia. Modes of germination in *Cookeina* species were studied by Paden (1975), Hanlin et al. (1991), Hanlin (1993) and Melendez-Howell (1986) and were found to conform to the original observation of Boedijn. The anamorphic states are similar to those of *Sarcoscypha* (Alexopoulos & Butler 1949, Harrington 1990, Rosinski 1953), *Phillipsia* (Paden 1975, 1984) and *Nanoscypha* (Pfister 1973).

Berthet (1964) stated that cultures were light in color, white or cream, never grey or black. He indicated that septal aureoles, thickenings associated with septa, are very well developed in cultures. Sánchez et al. (1993) and Sánchez Vázquez et al. (1995), investigating in Mexico the edible *Cookeina speciosa,* determined the conditions under which ascomata develop and the cultural requirements necessary for fruiting. Sánchez Vázquez et al. (1995) suggests this species causes a white rot which is in agreement with our general observations on the condition of wood associated with fruiting.

Distribution

Two species, *C. speciosa* and *C. tricholoma*, are pantropical but *C. speciosa* represents a species complex and there is variation within the complex (Weinstein et al. 2002). Other species seem to be more limited in distribution. *Cookeina colensoi* is primarily a southern hemisphere taxon occurring in subtropical zones. A few collections are known above the equator in Asia and the Americas. *Cookeina indica, C. insititia*, and *C. sinensis* are restricted to Asia. The range for *C. indica* has been expanded in recent years from India to Southwestern China (Yang 1997). In the Americas, *C. venezuelae* is known from northern South America, Central America and from the West Indies. Gamundí (1983) presented a distribution map for Central and South American species. In this paper *C. colensoiopsis* is described from Mexico and Venezuela.

Phylogeny and relationships

The genus *Cookeina* has been placed in the family *Sarcoscyphaceae* in modern treatments (Cabrello 1988, Eckblad 1968, Harrington et al. 1999, Korf 1970, 1972, 1973, Le Gal 1969, Weinstein et al. 2002). The family is recognized fundamentally in the sense of Korf (1970, 1972, 1973) as one of two families considered to have thick ascus walls and thickened opercular areas. The *Sarcoscyphaceae* are generally brightly pigmented and the *Sarcosomataceae* are generally dark in color. In all molecular phylogenetic studies a monophyletic *Sarcoscyphaceae* is recovered. Within the group, *Cookeina* and *Microstoma* form a well-supported clade. This group corresponds to the tribe *Boedijnopezizeae* as delimited by Korf (1970).

Weinstein et al. (2002) studied the species of *Cookeina* using ITS sequence data. Morphologically the species are distinguished by the combination of several features including ascospore shape and surface relief, presence and orgin of apothecial hairs and presence or absence of gelatinous material within the cortical layer of the excipular tissue. The genus was shown to be monophyletic with several well-supported lineages that correspond to the morphological species concept traditionally used. Collections referred to as *C. speciosa* segregate within a clade. Hymenial color differences correlate with groups within that clade. In this work we consider *C. speciosa* to represent a species complex. Detailed populational studies will be necessary to understand fully relationships within this complex. The placement of *C. insititia* in the ITS study is ambiguous but falls within *Cookeina*. Thus, the genus *Boedijnopeziza*, with *C. insititia* as the type species, is not recognized.

Taxonomic treatment

Cookeina Kuntze, Rev. Gen. Pl. 2:849. 1891.

[= *Trichoscypha* (Cooke) Sacc., Syll. fung. 8: 160. 1889, non Hook. f, 1862.]
= *Pilocratera* Henn., Bot. Jahrb. Syst. 17: 9. 1891.
= *Boedijnopeziza* S. Ito & S. Imai, Trans. Sapporo Nat. Hist. Soc. 15: 58. 1937.

TYPE SPECIES: *Peziza tricholoma* Mont. (selected Seaver, 1927)

Apothecia medium to large, cupulate to funnel-shaped, sessile or stipitate. **Hymenium** white, pink, yellow, orange, salmon, rose, or chocolate. **Outer surface** of the apothecium concolorous or lighter than the hymenium, nearly glabrous to tomentose or with long, prominent fasciculate hairs on the margin. **Excipulum** of two distinct layers: an inner layer of *textura intricata* of rather narrow diameter and an outer layer of pyriform to globose cells which arise from hyphae originating in the medullary excipulum. The outer layer is constructed of two zones, the inner zone of which may contain gelatinous material. **Asci** cylindrical with a prominently thickened lateral operculum, J–, eight-spored, at the base abruptly connected to a narrow, long, hypha, all spores within a single apothecium at the same state of maturation. **Ascospores** hyaline, smooth or with longitudinal and rarely transverse markings, ellipsoid to fusoid, pinkish or buff in deposit, often bilaterally asymmetrical, guttulate. **Paraphyses** anastomosing to form a network around the asci, in some species developing apically to form setiform hairs.

SUBSTRATE: On decaying, generally recently dead, twigs, branches, and larger logs.

DISTRIBUTION: Species are found throughout the lowland tropics. *C. tricholoma* and *C. speciosa* are pantropical. Other species have restricted distributions.

Key to species of *Cookeina*

1. Outer surface of the ascomata with distinct hairs that are visible without a handlens, with a well developed stipe 1
1. Outer surface of the ascomata lacking obvious hairs, lacking a stipe or with a short stipe 5

2. Hairs arranged prominently at the margin of the apothecium, hairs pyramidal in form, apothecia narrow goblet form, gel layer present in the excipulum, ascospores narrow sub-fusoid to fusoid, asymmetrical and distinctly curved, with pointed ends .... *C. insititia*
2. Hairs either grouped at the margin or more or less evenly distributed over the outersurface of the apothecium, apothecia widely flaring, ascospores ellipsoid to broadly fusoid 3

3. Hairs forming distinct rows or ridges at the margin of the apothecia, hairs arising from the medullary layer, ascospores ellipsoid .... *C. speciosa* complex
3. Hairs more or less evenly covering the outer surface of the apothecium, spores ellipsoid to broad fusoid 4

4. Ascospores smooth .... *C. sinensis*
4. Ascospores with longitudinal striations .... *C. tricholoma*

5. Excipulum lacking gelatinous material, ascospores elliptical to broadly fusoid with longitudinal striations, flattened at one side, appearing sub-papillate ... *C. indica*
5. Excipulum with gelatinous material 6

6. Ascospores ornamented with both longitudinal and transverse folds, ellipsoid, apiculate at each end, bilaterally symmetrical or asymmetrical....... *C. venezuelae*
6. Ascospores smooth (if considered short stipitate, *C. insititia* could key here) 7

7. Paraphyses without setose projections, ascospores ellipsoid to subfusoid, sometimes short apiculi or papillae present at poles .... *C. colensoi*
7. Paraphyses with setose projections, ascospores ellipsoid, smooth .... *C. colensoiopsis*

Descriptions of species

Cookeina colensoi (Berk.) Seaver, Mycologia 5: 191. 1913. **Fig. 3**

≡ *Peziza colensoi* Berk. in Hooker, Fl. nov.-zel. 2: 200. 1855 !! ≡ *Sarcoscypha colensoi* (Berk.) Sacc., Syll. fung. 8: 157. 1889. ≡ *Cookeina colensoi* (Berk.) Seaver, Mycologia 5: 191. 1913. [misapplied = *Cookeina venezuelae* (Berk. & M.A. Curtis.) Le Gal] ≡ *Boedijnopeziza colensoi* (Berk.) Korf & Erb, Phytologia 21: 202. 1971.

= *Peziza aluticolor* Berk., J. Linn. Soc., Bot. 13: 176. 1872 [1873] !! ≡ *Geopyxis aluticolor* (Berk.) Sacc., Syll. fung. 8: 64. 1889. ≡ *Ciboria aluticolor* (Berk.) Rick, Ann. Mycol. 2: 408. 1904.

= *Geopyxis moelleriana* Henn., Hedwigia 41:30. 1902.

= *Geopyxis ciborioides* Starbäck, Ark. Bot. 20 (2): 1. 1904. ≡ *Geopyxis aluticolor* var. *ciborioides* (Starbäck) Rick, Brotéria Sér. Bot. 25: 81. 1931. ≡ *Cookeina colensoi* var. *ciborioides* (Starbäck) Gamundí, Bol. Soc. Argent. Bot. 6: 218. 1957.

= *Ciboria sessilis* Starbäck, Ark. Bot. Utgivet. Avk. Svenska 2 (5): 3. 1904 !
= *Ciboria argentinensis* Speg., Anales Mus. Nac. Hist. Nat. Buenos Aires III 19: 444. 1909 !!, !
[= *Peziza subtropica* Speg. in ed., fide Gamundí (1959)]

Apothecia scattered, short stipitate centrally, seldom subsessile, deeply cup-shaped, when dry, 2–15 mm tall and 2–17 mm diam. **Disc** brighter colored than receptacle, when fresh, orange to orange-yellow, when dry, ochre, ochraceous or orange–ochraceous. **Margin** frequently in-rolled when dry, with 0–4 low circular ridges more evident when dry, with tomentum bundles originating from the outer excipulum. **Receptacle** when fresh, paler than disc, up to 25 mm tall and 2–20 mm diam; when rehydrated, orange to golden–yellow to yellow to light–yellow to cream; when dry, 10–17 mm diam, cream, light yellow, yellow, orange, ochre, beige, or brown; sometimes drying in a venose, convoluted, ribbed pattern, more evident at the base of the receptacle where it shows a ribbed or venous pattern, continuing as striations on the stipe; furfuraceus. **Stipe** short to substipitate, when fresh or re-hydrated light yellow to cream-colored to whitish and 2–20 x 1–3 mm, when dry yellow to orange, concolorous with dry receptacle and 2–15 x 1–2 mm, sub-cylindrical somewhat wider at the base with a disc-shaped "holdfast" reaching 2 mm diam, with longitudinal ridges and furrows when dry on all of its length and sometimes extending towards the receptacle. **Tomentum** of two types: 1) individual monilioid processes that arise from the margin and also sparsely covering all the receptacle giving it a furfuraceous appearance, composed of 3–5 short hyaline cells, cells 8–12 µm diam with thick and rugose walls, walls 2–4 µm wide, hairs 44–100 x 10–14 µm; 2) fused triangular-shaped bundles of cells present at the margin, 40–100 µm long, 34–64 µm wide at the base, and 8–22 µm wide at the apex. **Outer excipulum** of *textura primatica* to *textura angularis*, becoming globose on the outside, forming a 3–5 celled-layer of 20–40 µm thick, cells 8–14 x 8–16 µm, walls of outer cells roughened with brownish material, outer layer is irregularly thickened giving it a pruinose appearance. **Medullary excipulum** divided into two layers: the layer adjacent to the ectal excipulum of *textura oblita*, of thin-walled hyphae immersed in a gel and oriented perpendicular to the receptacle surface, (20-) 40–80 µm wide, hyphae 1–5 µm diam. The layer more proximal to the sub-hymenium of *textura porrecta* 60–90 µm thick, sometimes with gel in patches, long–celled, arranged parallel to the receptacle surface, cells 3–6 µm wide. **Subhymenium** *textura intrincata*, 20–40 µm wide, somewhat gelatinized. **Hymenium** 280–350 µm thick, with no setae present. **Asci** cylindrical, base round to slightly tapering, (252-) 340–416 x 16–24 µm, abruptly arising from thin basal hyphae 8–32 x 4–8 µm, 8-spored located in the top 1/2 of the ascus, **Ascospores** obliquely uniseriate, broad elliptic-fusoid narrowing at the poles, unequal with a flatter side, sometimes short apiculi or papillae present, (0-)2(-3) large oil guttules and often several smaller ones, hyaline, smooth, 24–40 x 9–16 µm. **Paraphyses** filiform, septate, braching, with frequent anastomosis forming a dense network, agglutinated, 1.6–4 µm wide in the middle, enlarging at the apex to 4 µm, equal to the asci or exceeding them by 2–4 µm.

Substrate: on twigs 6–10 mm diam, on decomposed wood.

Distribution: Australia, New Zealand, India, Madagascar, Samoa, China, Argentina, Brasil, Colombia, Jamaica, Mexico .

Illustrations: Hooker (1855) as *Peziza colensoi*, f. 5a-c; Cooke (1879) as *P. colensoi* fig 108, as *P. aluticolor* f. 198; Cooke (1892) as *Geopyxis aluticolor*, fig. 144; Massee (1896)

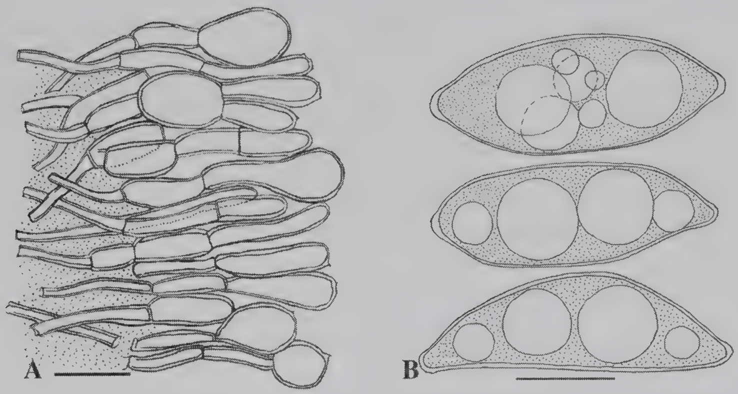


Figure 3. *Cookeina colensoi.* A. Cross section showing outer layer of the excipulum, bar = µm. B. Ascospores, bar = µm. New Zealand, Bay of Plenty, Kaimai Ranges, vic. Katikati, 15 Feb 1998, *S. P. Mortimer* (PDD *68535*).

tab. 16, fig. 14-15; Starbäck (1904) as *Geopyxis ciborioides*, f. 1–3; Starbäck (1904) as *Ciboria sessilis*, figs 8, 9; Le Gal (1953) figs. 110–113;

Gamundí (1957) Lam. I; Rifai (1968) figs. 21, 22; Chacón & Medel (1990) figs. 17-20; Hood (1992) fig. 14 a; Weinstein et al. (2002) fig. 3E.

Notes on type specimens and nomenclature: The holotype specimen of *C. colensoi* from Berkeley's herbarium (K) has a drawing of a the characteristic spore on the packet. The specimen was placed in a type cover by R. W. G. Dennis (Brian Spooner, pers. com.) although it is note labeled as completely as the prologue suggests (see specimens cited below). Massee (1896) cites Colenso, no. 2810. His drawing and description accurately depict the structure of the outer excipulum and may be the first to describe and illustrate the anatomy of this species. After a study of the holotype of *Peziza aluticolor* we agree with Cooke (1879) and Saccardo (1889) that this material should be referred to *C. colensoi*. Seaver (1928) used the name *C. colensoi* for a different species, *C. venezuelae,* as pointed out by Le Gal (1953). The type specimen of *Geopyxis ciborioides* (Brazil, Rio Grande do Sul, col. Ijuhy, ad ramos mucescentes humi jacentes in silva primaea, 4/4 1893, 323 B) was not located in our search. Gamundí (1957) also was unable to locate the type specimen and followed Rick (1931) in treating this as *G. aluticolor* var. *ciborioides*. Gamundí (1957) stated that *Geopyxis aluticolor* var. *ciborioides* differs from *Cookeina colensoi* only by the absence of paraphyses. In our experience, all species of *Cookeina* have paraphyses; we question the observation that they are absent in this taxon. We studied the holotype and isotype specimens of *Ciboria argentinensis* as did Gamundí, but found that Gamundí's (1957) measurements of ascospores and asci differed from ours, 24–28.8 x 8.4–9.6 µm vs. 24–32 x 8.8–13.6 µm for ascospores and 317–335 x 14.5–18.8 µm and 340–380 x 16–24 µm for asci. She stated that asci and ascospores of *C. argentinensis* are smaller than those found in other collections of *C. colensoi*. It is the case that asci and ascospores of *C. argentinensis* fall at the lower range for this species but they are not as small as she indicated. Based on the description, Le Gal (1953) thought *G. elata* was a synonym of *C. colensoi*, not of *C. sulcipes* as Boedijn

(1933) indicated. On reading the description, we can understand Le Gal's interpretation, since the evident marginal hairs and furrows of *C. speciosa* are not mentioned, though two of the characters that Massee (1898) describes are not at all typical of *C. colensoi*: the length of the stipe (6–8 cm) and its growing on the ground. We were able to examine the type specimen (NY) of this species, and determined it to be *C. speciosa*. It presents the following characters, typical of *C. speciosa*: marginal ridges (three in this specimen) with evident hairs, no gel in the excipular tissues, fine striations on the ascospores, and hymenial setae. It is likely that Massee's mention of this fungus growing on soil is based on material fruiting on buried twigs. We were unable to locate any of the original material of *Geopyxis moelleriana* (St. Cathar. bei Blumenau und Velha auf Holz, April 1891, 24 November 1891. No. 54b, 252, 903. Col. Möller). The holotype of *Ciboria sessilis*, from Brazil, consists on a few apothecia, all with short stipes (up to 5 mm), with the exception of one which has a stipe 15 mm long. The specific epithet "*sessilis*" may not have been particularly appropriate.

General Notes: This species is easily recognized because of its lack of hairs, presence of a gelatinous excipular layer and ascospores that appear smooth under the light microscope. The ascospore surface was shown by Weinstein et al. (2002) to be smooth under SEM examination; this conforms to the standard interpretation of the species. Moravec (1997) described the spore ornamentation under light microscopy as wrinkled and under SEM showed low, interconnected ridges and channels. His material was from Madagascar and has not been reexamined by us. Le Gal (1953) indicated that ascospores were smooth in the materials she examined from Madagscar. Neither Moravec nor Le Gal mentioned the presence of a gelatinous layer. As fresh material from Madagascar becomes available it might be worthwhile to reexamine it critically and to consider the possiblity that the Madagascar specimens represent a distinct, undescribed species.

There are several variable features among collections. In some specimens, the cells of both types of tomentum were covered with an amorphous material or deposition. These deposits give the cells a rugose appearance. There is variation in spore and ascus size. For example, a collection from Sao Leopoldo, Brazil, from the Lloyd herbarium (BPI) has spores 13–14 x 5–6 µm; these are smaller than we recorded but the other characters match well *C. colensoi*. Le Gal (1953) reported asci up to 440 µm, triangular hairs up to 160 µm tall and 100 µm wide at the base. These are larger than we found for these structures. Rifai's (1968) measurements of hairs are larger as well, 150 µm long and 80 µm at the base. But he reports asci and paraphyses that are thinner: asci 14–18 µm, paraphyses 1–1.8 µm. Korf & Erb (Korf 1971) transfered *C. colensoi* to *Boedijnopeziza* and later Korf (1973) used the character of presence of a gelatinous layer to distinguish the genus *Boedijnopeziza*. Examination of their material showed it to be *C. venezuelae* rather than *C. colensoi*. The gel layer as a character for distinguishing the genus *Boedijnopeziza* has been the subject of considerable debate over the years. For a more detailed discussion of the confusion surrounding *Boedijnopeziza* and gelatinous material see the introduction.

Specimens examined. ARGENTINA. BUENOS AIRES: Punta Lara, ad ramenta arborum sempervirentium in silva marginali, locis umbrosis, 9 Jun 1949, *R. Singer 2129* (CUP); TUCUMÁN: ramas muertas putrescentes, Celtis sp., 16 April 1906, *C. Spegazzini 4107* (LPS, HT with Spegazzini's drawings of asci, ascospores and paraphyses, IT (2 packets) of *Ciboria argentinensis*); Anta Muerta, Sierra de San Javier, on old fallen branches, 24 Apr 1949, *Singer & Digilio 2116* (CUP); Quebrada de Lules, ad ramos emortuos, 2 Apr 1949, *R. Singer 2114* (CUP); 35km del camino a Tafi del Valle, 26 Feb 1949, *R. Singer 2115* (CUP). AUSTRALIA. NEW SOUTH WALES: Clarence River, HT of *Peziza aluticolor* (K, NY-G. Massee Herb.); Bobong Creek, Wild Cattle Creek State Forest, near Cascade, on undetermined wood, 11 Feb 1984, R. Coveny 38/1984 (DAR 63642a); Dorrigo, Dorrigo National Park, on undetermined wood, 12 Feb 1984, R. Covey 56/1984 (DAR 63646a). BRASIL. MATO GROSSO: Sta. Anna de Chapada, in silva ad lignum. 23 Feb 1894, Madine 546 (B); HT of *Ciboria sessilis* Starbäck (S); *G. O. Malme,* 546B (S); NOVA PETROPOLIS: 1923, *Rick* (FH); SANTA CATARINA: Porto Novo, Sta. Catharina, 1928, *Rick 531* (FH) [as *Geopyxis aluticolor*]; Sao Leopoldo, in ramis frequentissima, 1903, *Rick 14* Austro-Americani (NY, FH, FH-Pat) [as *Ciboria aluticolor*]; Feb 1904 (FH) [as *Ciboria aluticolor*]; Sao Leopoldo, Rio Grande do Sul, 1925, *Rick* (FH) [as *Geopyxis aluticolor*]; Sao Leopoldo, *J. Dutra 33* (NY); S. Salvador, 4 Jan 1944, *Rick 20803* (NY) [as *Ciboria*], packet is empty. CHINA. GUIZHOU: on twigs, 4 Aug 1988, *Li Yu Zong, Yu-chen & Ying Jian-zhe 59537* ex HMAS (FH), Xishungbanna, Menlen, ex situ of endangerous plants area, 500 m. alt, on rotten wood. *M. Zang 11532*, 25 X 1988 (HKAS 20381). COLOMBIA. MAGDALENA: Sierra Nevada de Santa Marta, Cerro Quemado trail, 1500-2300m, 24 Aug 1935, *G. W. Martin 3714* (FH). INDIA. West Kameng, A. P., on wood in an angiospermous forest, 5 Sept 1981, *Rishi Kaushal 18556* (FH); as previous, *Rishi Kaushal 18557* (FH). MEXICO. TAMAULIPAS: Rancho del Cielo, on fallen limb, 29 Jul 1974, *A. J. Sharp 62500* [ex Herb. Univ. Tenn. 39652] (CUP 62500) (FH 39652). NEW ZEALAND. SOUTHERN NORTH ISLAND.: On dead sticks, near the River Manawatu, Colenso, holotype of *P. colensoi* (K), (NY-G. Massee Herb) [as *Peziza colensoi, Peziza aluticolor*]; AUCKLAND: Hunua Ranges, Cossey's Track, on wood, 9 May 1996, *1996 Fungal Foray* (PDD*66040*); BAY OF PLENTY: Kaimai Ranges, Timms Road, vic. Katikati, on decorticated wood, 15 Feb 1998, *S. P. Mortimer* (PDD *68535*); COROMANDEL: Kauaeranga Valley, vic. Thames, on decorticated wood, 1 Apr 1981, *G. J. Samuels & H. Thiers* (PDD *42052*); On decorticated wood, 1 Apr 1981, *G. J. Samuels, H. Thiers,* (PDD *42053*); Kopu-Tairua Road, Kaitarakihi Summit Track, 37 08'S, 175 41'E, on decorticated wood, 14 Mar 1998, *P. R. Johnston, R. E. Beever, S. L. Stephenson* (PDD *68628*); vic. Port Charles, track from Stoney Bay to Fletcher Bay, on wood, 25 Feb 1989, *P. R. Johnston* (PDD *55306*); NORTHLAND: Mangamuka Scenic Reserve, on wood, 8 May 1983, *G. J. Samuels, T. Matsushima, R. H. Petersen* (PDD *46278*); Omahuta S. F., Omahuta Kauri Sanctuary vic. Mangamuka Bridge, on wood, 10 May 1981, *G. J. Samuels, E. Horak* (PDD *42049*); WAIKATO: vic. Otorohanga, Native Forest Restoration Trust, Owen Lewis Reserve, 220m, on blackened, decorticated wood, 24 May 2000, *P. R. Johnston* (PDD *71534*); Waitomo, on bark and decort. wood, 26 Apr 1983, *G. J. Samuels, P. R. Johnston, R. H. Petersen* (NY, PDD *46838*). SAMOA. *C. G. Lloyd 5020* (FH-Pat) [as *Peziza (Geopyxis) aluticolor*]; Jan 1900, *C. G. Lloyd 5020* (NY); 1904-5, *C. G. Lloyd 5021* (NY), infertile.

Cookeina colensoiopsis Iturriaga & Pfister, **sp. nov.** Fig. 4

Haec species C. colensoi *similitudit ex strato gelatinoso, tomento et ascosporis laevibus adest, sed haec differt eo setas in hymenio habet. Holotypus: Cerro El Avila, on wood, Norte de Caracas, Agosto 2002,* Tamia Souto (FH)

Apothecia scattered, centrally stipitate, deeply cup-shaped, up to 20 mm tall when fresh, and 14–15 mm tall when dry. **Receptacle,** when fresh, 17 mm tall and 2–15 mm diam, when dry 6–8 mm tall and 15–20 mm diam; when fresh lighter colored than the disc, when rehydrated, light yellow receptacle and margin; when dry, beige; drying in a venose–ribbed pattern at the base, which continues with striation in the stipe, furfuraceous uniformly because of white hair-like projections. **Stipe** cream-colored (fresh), sub-cylindrical, somewhat wider at the base, 3-13 x 1-3 mm (fresh) and up to 2 x 9 mm (dry), longitudinally ribbed when dry, furfuraceous with white hair-like projections. **Disc** darker colored than receptacle, bright yellow to orange (fresh), ochre (dried), orange (rehydrated). **Margin** concolorous to receptacle when fresh or dry, ribbed with three striations or ridges, presenting the two types of tomentum. **Tomentum** of two types, originating from the outer excipulum: 1) individual monilioid processes, minute, as hair-like projections, covering margin, receptacle and stipe, giving it a furfuraceous appearance, 30–92 x 10–24 μm, 2–10 cells tall, cells round or elongated with thick-rugose walls, cells 10–22 x 8–16 μm; 2) short and whitish triangular-shaped bundles consisting of 5–12 fused individual monilioid *gosto 2002, Tamia Souto (FH).* processes, only present on marginal ridges. **Outer ectal excipulum** of *textura globulosa* to *textura prismatica*, 4–5–celled layer 30–90 μm wide, cells 8.0–14 μm diam. **Medullary**

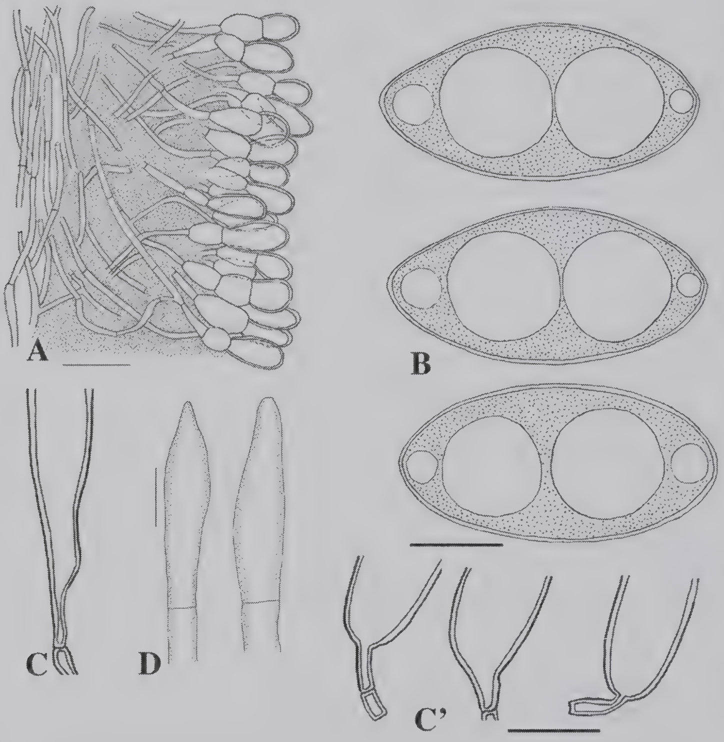


Figure 4. *Cookeina colensoiopsis.* A. Cross section showing outer layer of the excipulum, bar = 25 μm. B. Ascospores, bar = 10 μm. C, C'. Ascus bases, bar = 25 μm. D. Setose apices of paraphyses, bar = 25 μm. Caracas, Venezuela; on decaying wood, 1903, *A. F. Blakeslee* (FH).

excipulum divided into two layers: the layer proximate to the ectal excipulum of *textura oblita*, thin–walled hypha immersed in a gel and oriented outwards perpendicular to the receptacle's surface, layer 50–100 µm wide, hyphae 2–4 µm diam. The layer proximate to the sub-hymenium (or more distant to the ectal excipulum) of *textura porrecta*, long–celled, layer 60–100 (–600) µm wide, cells 4–6 µm wide. **Subhymenium** *textura intricata*, somewhat gelatinized, layer 4550 µm wide. **Hymenium** 380–450 µm thick. **Hymenial setae** present, intemixed with asci and paraphyses, brownish, 430–440 x 2 µm, with lanceolate terminal cell, exceeding hymenial elements by 10–20 µm. **Asci** long cylindrical with a round base, 340–420 x 10–20 µm, ending in an abrupt manner to connect with hyphal–like basal appendage, a few times tapering slightly at base, basal appendage 4–28 x 4 µm. **Ascospores** obliquely uniseriate broad elliptic, unequally sided, narrowing slightly at ends, with a short apiculum present at both ends, mostly 2 large central oil guttules with two smaller ones at ends, hyaline, smooth walled, with no wall markings, 30–34 x 12–14 µm. **Paraphyses** filiform, septate, branching, anastomosing to form a network, agglutinated, 2–4 µm wide in the middle, enlarging in a clavate shape at the apex to 4.0 µm.

SUBSTRATE: on decaying wood.

DISTRIBUTION: known from Venezuela, possibly only from El Ávila National Park.

GENERAL NOTES: Our attention was caught by an unusual specimen identified as *C. colensoi*. Denison (1967) studied this collection and wrote that "a single collection [of *C. colensoi*] is known from Venezuela, which suggests that it may yet be found in Central America." In order to determine if this specimen really belonged to *C. colensoi*, it was re-examined and found to differ in form of the paraphyses from *C. colensoi*. This specimen was collected by A. F. Blakeslee in 1903, in Caracas, Venezuela, on decaying wood (FH). Specimen annotations by Blakeslee indicated that "it is not *P. venezueliana* [sic]" and that ascospores are 40–45 x 14–16 µm (our measurements from the same specimen were 30-34 x 12-14 µm). The species was known only from that single collection from the Caracas region, and we do not know the exact locations Blakeslee visited. Since the Caracas area is reasonably well collected and in view of the urban expansion, we assumed that the species no longer existed. Those initial conclusions proved to be wrong. In August 2002 a *Cookeina* specimen collected from Parque Nacional El Avila massif, located at the northern side of the city of Caracas was brought to the senior author for determination. It proved to be this species that we have now described as *C. colensoiopsis*.

Measurements from the recent collection (Paratype: PT) are larger than those from Blakeslee (HT): outer ectal excipulum layer 40–50 µm wide (HT), 30-90 µm (PT); inner ectal excipulum gelatinized, 30-40 µm widte (HT) and 50-100 µm (PT); medullary excipulum non-gelatinized 60-100 µm wide (HT) and 470-600 µm (PT); asci up to 360 µm (HT) and to 420 µm (PT).

This species resembles *C. colensoi* in the presence of a gel layer, tomentum and smooth ascospores, but differs from it in having hymenial setae. Such setae were observed previously only in *C. speciosa*.

SPECIMENS EXAMINED. VENEZUELA. FEDERAL DISTRICT: Caracas, on decaying wood, 1903, *A. F. Blakeslee*, Det.: D. H. Linder. [as *Cookeina colensoi*] (FH). Holotype; Cerro El Avila, on wood, Norte de Caracas, Agosto 2002, Tamia Souto (FH).

Cookeina indica Pfister & R. Kaushal, Mycotaxon 20: 117. 1984 !!, ! **Fig. 5**

Apothecia scattered to gregarious, cupulate, disc-shaped when dry, centrally to slightly eccentrically stipitate, sub-stipitate, or sessile, with a narrow point of attachment, up to 35 mm high and up to 30 mm in diam, tough in consistency. **Receptacle** concolorous with the disc, yellow when fresh, when dry, orange to blackish, light ochre when re-hydrated, nearly smooth except at the margin. **Stipe,** when present, solid, terete, whitish, smooth, up to 22 mm long and up to 1.5 mm thick, short or often reduced to a narrow point of attachment of 1 x 1 mm. **Disc** deeply concave, yellow when fresh, smooth. **Margin** entire, minutely tomentose. **Tomentum** as hair-like processes arising from the cells of the outer excipulum, composed of up to 4 round cells, 40–80 × 10–14 µm. **Ectal excipulum** of *textura angularis* to *textura globulosa*, of 3-6 cell layers, 40–50 µm thick, cells 10–25 × 14.5–25 µm. **Medullary excipulum** divided into two layers: the layer proximate to the ectal excipulum of *textura porrecta*, long and parallel, without a gel. The layer proximate to the subhymenium of *textura intricata* to *textura porrecta,* up to 160 µm thick, of interwoven hyphae in a more or less parallel arrangement at the junction between the medullary and outer layers, 4–6 µm diam. **Subhymenium** of *textura intricata* of loosely interwoven septate and branched hyphae 2–2.7 µm diam. **Asci** long cylindrical, apices obtuse, at the base usually abruptly contracted or attenuated into a narrow basal hyphal extension, 300–370 × 14–20 µm, thick-walled, with 8 ascospores. **Ascospores** ellipsoid to broad elliptic-fusoid to fusiform in face view, in side view, flattened at one side narrowing towards the poles, in both views appearing sub-papillate; wall 1.0–1.5 µm, thicker at the poles; hyaline, ornamentation of fine longitudinal, parallel ridges approximately 1 per µm that sometimes anastomose; guttules arranged in four possible ways: 1) with 3 guttules, 2) 1–2 central guttules with smaller surrounding guttules, 3) two large central guttules, two of middle size accompanied by smaller ones toward the poles, or 4) numerous guttules; (18-) 26.5–40.0 (–47) × 10–15 (–17) µm, usually obliquely uniseriate. **Paraphyses** thread-like, delicate, septate, frequently anastomosing, sometimes constricted at septa, (–2.5) 3-4 (–4.5) µm in the middle, branching more often at the apex, and presenting varied types of apices, sometimes several types can be present in one apothecium: 1) swollen-clavate apices up to 5 µm. 2) mucronate apices with 5 µm at its widest diameter ending in a somewhat pointed apex of 1 µm diameter, or 3) irregular up to 4 µm wide; the mucronate type apices (2) projects slightly beyond the ascus tips.

SUBSTRATE: On angiospermous wood.

DISTRIBUTION: India, West Kameng (holotype and paratype locality), and China, Yunnan (Yang 1990).

ILLUSTRATIONS: Pfister & Kaushal (1984), fig 1; Weinstein et al. (2002), fig. 3B.

NOTES ON TYPE SPECIMENS AND NOMENCLATURE: Specimens examined are mostly those studied by Pfister & Kaushal (1984). No specimens of *Cookeina mundkurii* S. C. Kaushal have been available for study but we suspect it is *C. indica.*

GENERAL NOTES: *Cookeina indica* has narrow ellipsoid to fusoid, often inequilateral, ascospores that are narrower than those found in either *C. sulcipes* or *C. tricholoma,* the other species with longitudinally striate ascospores. The striations in *C. indica* form more or less continuous bands on the spores. This species is characterized further by having a smooth receptacle, with only minute marginal hair-like projections. There is no

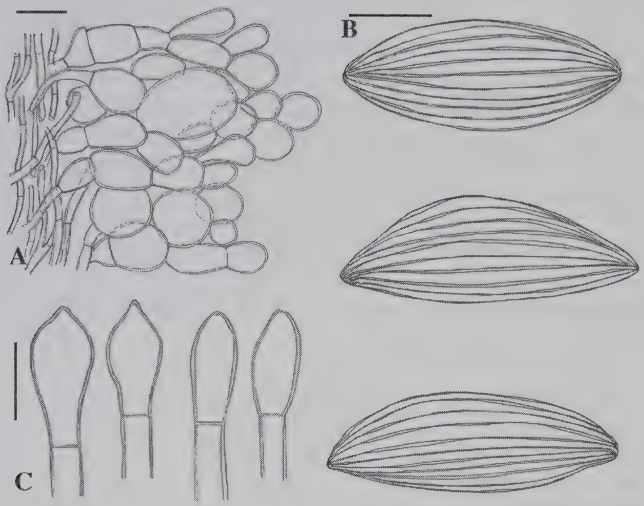


Figure 5. *Cookeina indica.* A. Cross section showing outer layer of the excipulum, bar = 25 µm. B. Ascospores, bar = 10 µm. C. Apices of paraphyses, bar = 10 µm. Yunnan, China; 5 Nov 1999, *119* (HMAS).

gelatinous material in the ascomata. A distinctive feature for this species is the variety of shapes of the apical cells of the paraphyses. Mucronate and irregular cell shapes such as these are only present in this species. The typical filiform, slightly clavate, paraphyses found in all species of the genus and are also present in *C. indica*. Yang (1990) suggested that Teng (1963) reported *C. indica* under the name *C. colensoi*.

Specimens examined. CHINA. YUNNAN: On wood, 5 Nov 1999, *119* ex HMAS (FH); On wood, 5 Nov 1999, *119* (HMAS). INDIA. West Kameng, A. P. [Arunachal Pradesh], Tipi, on wood in an angiospermous forest, (alt. 300 m), 16 Sept 1981, *Rishi Kaushal 18611*, Holotype (FH); Photo of isotype specimen [as *Cookeina insititia*] at PAN (FH) (not examined at this time previously examined by Pfister & Kaushal (1984)); Dehra, on *Dalbergia* sp., 2 Sept 1952, *K. S. Thind 572400*, Paratype (BPI) [as *Cookeina colensoi*] slide made from Paratype specimen (FH).

Cookeina insititia (Berk. & M.A. Curtis) Kuntze, Revis. gen. pl. 2: 849. 1891. **Fig. 6**

≡ *Peziza insititia* Berk. & M.A. Curtis, Proc. Amer. Acad. Arts 4: 127. 1860 !, !! ≡ *Trichoscypha insititia* (Berk. & M.A. Curtis) Sacc., Syll. fung. 8: 161. 1889. ≡ *Pilocratera insititia* (Berk. & M.A. Curtis) Sacc. & Traverso, Syll. fung. 20: 412. 1911. ≡ *Boedijnopeziza insititia* (Berk. & M.A. Curtis) S. Ito & S. Imai, Trans. Sapporo. Nat. Hist. Soc. 15: 58. 1937. ≡ *Microstoma insititia* (Berk. & M.A. Curtis) Boedijn, Sydowia 5: 212. 1951.

=*Trichoscypha magnispora* Lloyd, Mycol. Writings 6: 1050. 1921 !

=*Boedijnopeziza sphaeroidospora* Y. Otani in Kobayasi et al., Bull. Natl. Sci. Mus. 14(3): 407. 1971 !

Apothecia scattered to gregarious, centrally stipitated, 9–25 mm tall when dry, mostly not exceeding 1 cm in length when fresh (fide Boedjin, 1933). **Disc** deeply concave, white to pale cream, when dry concolorous or lighter than dry receptacle. **Receptacle** deep-cup shaped, infundibiliform, urceolate to turbinate, seated on a well defined stipe, white to whitish, to pale cream colored, paler than the disc; when dry beige or yellow-ochre, 5–12 mm tall and 4–11 mm diam. **Margin** sometimes flesh-colored or concolorous with the receptacle, with white, erect, triangular-flattened hairs. **Stipe** terete whitish, when dry 3–16 mm long by 1–2 mm diam, when fresh up to 40 mm long fide Boedjin (1933), drying in a venose pattern due to contraction of the gel, covered by tomentum, sometimes with a disc-shaped point of attachment. **Hairs** of three types, all originated from outer ectal excipulum cells: 1) marginal twisted (when dry) hairs, light yellow, triangular and flattened, in 1–2 rows, up to 2 mm long and 0.5 mm at the base and gradually tapering towards the pointed apex; when young these hairs totally cover the hymenium in a nearly continuous sub-conical sheath, which later opens as the disc expands, splitting into 4–5 separate compound hairs positioned in an imbricate fashion then dividing at maturity in to many compound hairs, each formed by bundles of parallel, septate, unbranched, sub-hyaline hyphae; 2) hairs, straight, in the margin intermixed with the twisted hairs, and covering mainly the upper part of the receptacle, though some cover the receptacle, flat, half the size of the marginal ones, but similar in structure to them, composed of bundles of parallel, septate, unbranched, sub-hyaline hyphae 4–9 µm diam, turning wider and shorter, up to 14 µm wide, at the base of the bundle. 3) a tomentum composed of hyphal projections of th outer cells of the excipulum covering receptacle and stipe. **Ectal excipulum** composed of two layers: **Outer ectal excipulum** *texturaglobulosa* to *angularis*, layer of 30–50 µm thick, composed of two to four or sometimes more layers of globose, sub-globose or rarely polygonal cells 9–24 µm diam; the most external cells are globose, up to 28 µm diam, with thick and sometimes warted walls 2–3 µm wide. In some cases these cells aggregate to form masses of loosely connected cells, which appear as irregular small projections at surface view. **Inner ectal excipulum** of *textura oblita*, of loosely interwoven, delicate, septate, branched, thin-walled hyphae immersed in a distinct gelatinous matrix and oriented perpendicular to the receptacle's surface, layer (20–)40–80 µm thick,hyphae 1.0–5.0 µm diam. **Medullary excipulum** of *textura porrecta* to *intricata*, of parallel hyphae running parallel to the outer surface of the receptacle, 1.8–6.3 µm diam, septate, typically unbranched and becoming intricate near the margin. **Subhymenium** of *textura intricata*, composed of loosely interwoven septate and branched hyphae 2–2.7 µm diam. **Hymenium** about 390 µm thick. **Asci** cylindrical, tapering to a long thin obconical base, 400–453 x 12–16 µm, thick-walled, wall 1.5–2 µm wide, with 8 ascospores. **Ascospores** usually obliquely uniseriate, narrow sub-fusoidal to fusoidal, asymmetrical or distinctly curved, with pointed ends, hyaline, smooth-walled under light microscope, containing numerous guttules, 36–47(–52) × 8.0–16 µm. **Paraphyses** filiform, delicate, septate, branched and irregularly anastomosing, hyaline, 1–2.0 µm diam, sometimes with irregular swellings; their apices profusely divided forming a small number of short branches, 1.5 µm diam, forming a dense irregular network. SUBSTRATE: on wood, on decomposed wood.

DISTRIBUTION: China, Indonesia, Japan, Phillipines, Samoa.

ILLUSTRATIONS: Berkeley & Broome (1875) as *Peziza insititia*, tab. 5, fig 21; Boedjin (1933) figs. 2D–E, 3F, I; Lloyd (1921) as *Trichoscypha magnispora*, plate 179, fig. 1950;

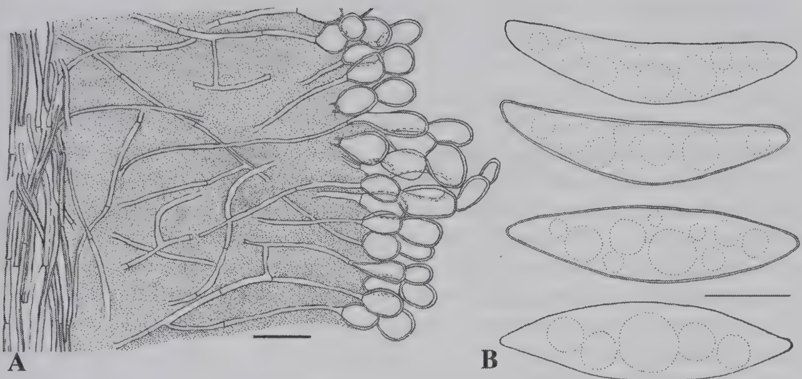


Figure 6. *Cookeina insititia*. A. Cross section showing outer layer of the excipulum, bar = 20 µm. B. Ascospores, bar = 10 µm. Yunnan, China; 17 Oct 1980, *Zang Mu* (CUP-CH 2527, HKAS 7240).

Massee (1896) as *P. insititia*, tab. 16, fig. 26–27; Melendez-Howell et al. (2003) figs. 8A–G, 9A–B, 10A–C and 11A–G; Otani (1971) figs. 17, 18; Rifai (1968) fig 23–25; Seaver (1928) fig 17i; Weinstein et al. (2002) fig. 3G.

Notes on type specimens and nomenclature: Both the holotype (K) and the isotype (FH-Curt.) of *Peziza insititia* were examined. Charles Wright collected the specimens on the U.S. North Pacific Exploring Expedition under Commanders Ringgold and Rodgers, but they have slightly different data on their labels. This is not unusual because of labeling for distribution. A primary set of specimens was given to Asa Gray who sent specimens to Curtis who in turn sent portions of them to Berkeley. Often only abbreviated information was provided to Berkeley. The holotype (K) bears the number 148 on the packet, which is the species number as originally listed for *P. insititia* by Berkeley & Curtis. Other data given are "on dead wood, Bonin Islands" but no further data on substrate or specific collecting date it states 1853-1856. The isotype (FH-Curt.) bears a more precise collecting date (Oct. 1854) and information on substrate (ad lign. inter folia dejecta), as well as the number 367 (C. Wright's collecting number). Wright and Small collected in the Bonin Islands between October 19 and November 1, 1954 (Pfister, 1978b). Berkeley & Curtis indicate as well that this species is allied to *P. tricholoma* Mont.

Ito & Imai (1937) erected *Boedijnopeziza* as a separate genus for *C. insititia* because of two features: the presence of a gel layer in the excipulum and smooth ascospores. The genus *Boedijnopeziza* is still a question of debate, being accepted by some investigators, such as Rifai (1968), Korf (1971, 1972, 1973) and Otani (1972) and not by others as Seaver (1928), Le Gal (1953), Denison (1967), Eckblad (1968), Pfister (1973), and Weinstein et al. (2000). Boedjin (1951) did not agree on the placement of *C. insititia* in the genus *Boedjinopeziza*, and considered it a member of the genus *Microstoma* stating that it had the characters of that genus: a gel layer, large fusoid spores, and anatomosing paraphyses. More recently a subgenus *Bedijnopeziza* was created in *Cookeina* for *C. insititia* (Melendez-Howell at al. 2003). This was based on SEM and TEM data related to wall layering and formation of ascospores and asci. It should be pointed out that Melendez-Howell used original material collected by Wright in the Bonin Islands (PC) in 1854 for their study. This appears to be an isotype. *Cookeina* and *Microstoma* resolve as sister groups in several analyses (Harrington et al. 1999). Weinstein et al. (2002) used *Microstoma* as an outgroup in their study of *Cookeina*.

Boedijnopeziza sphaeroidospora (Otani 1971) was studied by DHP (unpublished) who concluded that the type specimen was an immature specimen of *Cookeina institia*, and hence the round ascospores were immature. Otani (1975) came to the same conclusion after studying a series of developing apothecia of *C. insititia* in the field.

General Notes: We have not seen fresh material of this species; data on fresh specimens has been incorporated from Boedjin (1933), Rifai (1968), and Durrieu et al. (1997). The spores are smooth when viewed with the light microscope but they show low convolute markings under SEM (Melendez-Howell et al. 2003). Bi et al. (1993) mention that some spores are faintly ribbed but we have not seen such markings. It had been thought that among all the other species in the genus, a unique characteristic of *C. insititia* was the presence of a single ascospore wall layer (Boedjin, 1933; Pfister 1973). Melendez-Howell et al. (2003) studied the wall composition of ascospores of *C. insititia, C. speciosa* (as *C. sulcipes*) and *C. tricholoma*. The "paroí propre," or proper wall together with the perispore, are the two layers seen in all the ascospores of the *Cookeina* species studied by them. They showed that the structure of the "paroi propre" in *C. insititia* is less complex than in the other two species. The difference between species is not in the number of wall layers (as was previously thought), but in the complexity of the "paroí propre." The three species all have proper wall and a perispore, which are similar in thickness in the three species. The proper wall is more complex with at least three different layers in *C. tricholoma* and *C. speciosa* than in *C. insititia* (Melendez-Howell et al., 2003). Melendez-Howell et al. (2003) found the ascus apex to differ from the other species of *Cookeina* and the absence of the d2 wall layer distinguishes *C. insititia* from the other species. We do not find compelling evidence for the segregation in a separate genus of *C. insititia* particularly in light of the phylogenetic study (Weinstein et al. 2002).

Specimens examined. CHINA. HAINAN: Chang-Kiang, 12 Nov 1934, *S. Q. Deng 6374* (CUP-CH 1386); Tan-hsien, 24 Oct 1943, *S. Q. Deng 5564* (CUP-CH 1363); as above, 30 Oct 1934, *S. Q. Deng 5790* (CUP-CA 620); Ting-an, on wood, 18 Nov 1934, *S. Q. Deng 6681* (CUP-CH 622); as above, 13 Nov 1934, *S. Q. Deng 6453* (CUP-CH 621). YUNNAN: Hsichung Panna Autonomous, Prefecture, of Tai Nationality Region, Monlin, Nature Reserve, on rotten wood, 17 Oct 1980, *Zang Mu* (CUP-CH 2527, HKAS 7240). INDIA: Photo of India specimen at PAN (FH). INDONESIA. JAVA: Somarang, *Di van Leeuven 82* (NY). JAPAN. BONIN ISLANDS: On dead wood, C. Wright 148, U. S. Pacific Exploring Expedition, [holotype of *Peziza insititia*] (K); ad lign. inter folia dejecta, Oct. 1854, U.S. Pac. Ex. Ex., C Wright (367) (FH, FH–Curt.), [isotype of *Peziza insititia*] (FH-Curt.); 6 Oct 1915, *A. Yasuda 332* [very fragmented] (NY); Titizimia Island, Renzyudani, 23 Nov 1936, *Y. Kobayasi 664* (CUP-JA 664). PHILIPPINES: Six collections without collection data in OSC, *W. C. Denison*; On dead wood, *Denison 3941* = OSC 24420; *Denison 3942* (OSC 67796, 24419); Laguna, Los Baños, on dead wood, 24 Oct 1921, *Colin G. Welles 11671* (NY); Luzon, Mt. Maguiling, on dead wood, 26 Oct 1920, *A. Abesanis 10460* (FH-Pat); Oct 1920, *O. A. Reinking 10252* (FH-Pat); 28 Sept 1920, *O. A. Reinking 28341* (BPI-Lloyd) [holotype of *Trichoscypha magnispora*]; 25 Oct

1920, *P. Malabassan 10447* (FH-Pat); Bois pourris de arbres, 19 Oct 1887, *Balansa 43* (FH-Pat); Palo, on fine damp sticks, Jan 1906, *A. D. E. Elmer 7205* (NY). SAMOA: 1904-5, *C. G. Lloyd 5019* (NY).

Cookeina sinensis Z. Wang, Mycotaxon 62: 293. 1997 !!

Apothecium solitary or scattered, cupulate, centrally stipitate, up to 25 mm high and 50 mm diam when dry. **Receptacle** concolorous or paler than the disc, cinnamon-buff when dry, covered more or less uniformly with conspicuous long hairs. **Stipe** short, when rehydrated 8 x 1–2 mm and brownish-orange to cinnamon-buff, concolorous with receptacle, sub-cylindrical, somewhat wider at the base, with longitudinal ridges and furrows when dry over its length and sometimes extend to the receptacle. **Disc** deeply concave, pinkish, pinkish orange, buff to salmon, ochraceous-orange when dry. **Margin** with somewhat inrolled. **Hairs** fasciculate, white to brownish, arising from the medullary excipulum, composed of bundles of parallel, septate, thick-walled hyphae, stiff, bristle-like, 3–7 mm long, individual hyphae 6–8 µm diam, walls 1.5–2.5 µm wide. **Tomentum** not seen. **Outer excipulum** of *textura globulosa*, layer (50–) 80–175 µm wide, cells arranged perpendicularly to the surface of the receptacle, 8–24 µm diam, cells thick-walled, particularly the most external ones, walls 1.5–2.5 µm thick, hyaline. In some cases these cells aggregate to form masses of loosely connected cells, which gives the receptacle a pruinose surface. **Inner ectal excipulum** a thin layer of loose *textura porrecta* to *intricata* of thin–walled hyphae, layer 64–80 µm wide, hyphae 4–11 µm diam, no gel present. **Medullary excipulum** of *textura intricata*, 230–300 µm thick, hyphae septate, 2.5–10 µm wide. **Subhymenium** of *textura intricata*, indistinguishable from the medullary excipulum, 20–40 µm wide. **Hymenium** 500–525 µm thick, easily separated from the excipular layer. **Asci** cylindrical, long, 280–387 x 16–20 µm, narrow-hyphoid at base, thick-walled, 2–3 µm thick, 8 ascospores. **Ascospores** broad sub-fusoid to lemon-shaped, pointed at both ends, pale yellow when mature, smooth-walled, 0- to biguttulate, 25–40 x 12–17 µm. **Paraphyses** moniliform, slender, septate, branched and anastomosing, 2.5–4 µm.

SUBSTRATE: On dead twigs and debris.

DISTRIBUTION: Only known from China.

ILLUSTRATIONS: Wang (1997) fig. 2.; Wang (2001) fig. 1; Weinstein et al. (2002) fig. 3F.

NOTES ON TYPE SPECIMENS AND NOMENCLATURE: The designated holotype from HMAS 70088 has no ascospores. The type specimen being infertile, **we designate here an epitype, HKAS 14679, a paratype collected in the same area.**

GENERAL NOTES: *Cookeina sinensis* is very similar to *C. tricholoma*, the difference being that the ascospores of *C. sinensis* are smooth, and those of *C. tricholoma* are striate. There are apparently few collections of this fungus and its range to date is limited to China, including Taiwan (Wang 2001). We were only able to obtain three collections on loan. Molecular data show it is part of a larger group that contains *C. tricholoma,* but it appears to be distinctive from *C. tricholoma* (Weinstein et al., 2002).

SPECIMENS EXAMINED. CHINA. YUNNAN: MENLEN: Xishuangbanna: On twig, 8 Jun 1986, *Li Yu 372* [70088] (HMAS) without ascospores, holotype of *Cookeina sinensis*; as above, *72003* (HMAS); Limestone Hill, 1200 m, on rotten wood, 15 Aug 1985, *Zang Mu 10398,* 14679 (HKAS) [epitype].

Cookeina speciosa (Fr. : Fr.) Dennis, Mycotaxon 51: 239. 1994. Figs. 1 C-E, 7

≡ *Peziza speciosa* Fr., Syst. Mycol. 2: 84. 1822 [Type is illustration by Plumier cited by Dennis (1994) as listed above.] !

= *Peziza sulcipes* Berk., London J. Bot. 1: 141. 1842. [Type specimen: Surinam Hostin. (K).] infertile, no spores] ! ≡ *Trichoscypha sulcipes* (Berk.) Sacc., Syll. fung. 8: 161. 1889. ≡ *Cookeina sulcipes* (Berk.) Kuntze, Revis. gen. pl. 2: 849. 1891. ≡ *Pilocratera sulcipes* (Berk.) Sacc. & Traverso, Syll. fung. 20: 413. 1911.

= *Peziza hindsii* Berk., London J. Bot. 1: 456. 1842. [Type specimen: on dead wood, New Ireland, July] (K!) ≡ *Lachnea hindsii* (Berk.) Pat., Bull. Soc. Mycol. France 4: 98. 1888. ≡ *Trichoscypha hindsii* (Berk.) Sacc., Syll. fung. 8: 161. 1889. ≡ *Cookeina hindsii* (Berk.) Kuntze, Revis. gen. pl. 2: 849. 1891. ≡ *Pilocratera hindsii* (Berk.) Henn., Hedwigia 32: 225. 1893.

= *Peziza* (*Aleuria*) *javanica* Nees ex Lév., Ann. Sci. Nat. Bot. Ser 3 3: 39. 1845. [Type specimen: Java, ad palmas, herb. Blume] PC! ≡ *Trichoscypha javanica* (Nees ex Lév.) Sacc., Syll. fung. 8: 162. 1889. ≡ *Cookeina javanica* (Nees ex Lév.) Kuntze, Revis. gen. pl. 2: 849. 1891. ≡ *Aleuria javanica* (Nees ex Lév.) Overeem & D. Overeem, Bull. Jard. Bot. Buitenzorg. III 4: 12. 1922.

= *Peziza* (*Lachnea*) *amoena* Lév., Ann. Sci. Nat. Bot. III 3: 39. 1845. [Type specimen: Guiana ad truncos] ≡ *Trichoscypha amoena* (Lév.) Sacc., Syll. fung. 8: 161. 1889. ≡ *Cookeina amoena* (Lév.) Kuntze, Revis. gen. Pl. 2: 849. 1891.

= *Peziza afzelii* Fr., Nova Acta Regiae Soc. Sci. Upsal. III 1: 121. 1851. [type specimen: ad terra in Guinea, not found at UPS.] ≡ *Trichoscypha afzelii* (Fr.) Sacc., Syll. fung. 8: 161. 1889. ≡ *Cookeina afzelii* (Fr.) Kuntze, Revis. gen. pl. 2: 849. 1891. ≡ *Pilocratera afzelii* (Fr.) Sacc. & Traverso, Syll. fung. 20: 412. 1911.

= *Peziza (Geopyxis) hindsii* var. *beccariana* Ces., Atti Accad. Sci. Fis. 8 (4): 11. 1879. [Type specimen: lignicola, Ceylon] ≡ *Trichoscypha hindsii* var. *beccariana* (Ces.) Sacc, Syll. fung. 8: 162. 1889.

= *Pilocratera engleriana* Henn., Bot. Jahrb. Syst. 14: 363. 1892. (tab. 6, fig. 9.) (!) [Lectotype: designated here because holotype lost, Kamerun, Zenker (B)] ! ≡ *Trichoscypha engleriana* (Henn.) Sacc., Syll. fung. 11: 398. 1895.

= *Geopyxis elata* Massee, Bull. Misc. Inform. 138: 123. 1898! [Type specimen: On the ground, Kumusi River, W. Fitzgerald, New Guinea (K)!]

= *Pilocratera maxima* P. Syd., Ann. Mycol. 10: 82. 1912. [Type specimen: ad ligna vel ramos(?), Süd–Ost–Borneo. Hayoep, 18. 6. 1908, leg H. Winkler no. 2531]

= *Pilocratera novoguianensis* Ramsb. in Gibbs, Fl. Arfak Mts. p. 185. 1917. [Holotype: In ligno putrido. Manokoeari, sec. jungle, edge of "korang" forest, 200’. Jan 1914, Gibbs 6152] !

= *Cookeina sumatrana* Boedijn, Recueil Trav. Bot. Néerl. 26: 407. 1929. [Type specimen: auf vermoderten Baumstammen im Walde zu Soengai Pantojer (Deli) but a specimen is not particularly noted] = Have not examined type. Le Gal (1953) has it as a synonym of *Cookeina sulcipes.*

[= *Geopyxis striatospora* Maubl. & Roger, Bull. Soc. Mycol. France 52: 83. 1936 (teste Le Gal, 1953). Nom. inval. No Latin description or diagnosis given] [no specimen in PC] [Type specimen: leg. A. Mallamaire, Cote–d'Ivoire, 1934]

= *Cookeina sulcipes* var. *fusca* Alas., Nova Hedwigia 23: 771. 1972. [Type specimen: U.I.B/ L. 176 but this seems to refer to a series of collections from 1963 and 1964].

= *Cookeina globosa* Douanla-Meli, Mycotaxon 92: 225. 2005.

Apothecia solitary or clustered, centrally stipitate to rarely subsessile, deep cupulate to goblet shaped, leathery, becoming wrinkled with age, 10–50 mm broad, 12–80 mm tall.

Receptacle cup shaped or rarely funnel shaped, sometimes whitish, minutely scurfy except around the margin where there are usually up to 5 distinct concentric ridges from which compound hairs arise in mature specimens, in young specimens the ridges are absent and the hairs are arranged around the margin as a single row, variable in color, concolorous or much paler than the disc; when rehydrated light-brown, light-yellow, light yellowish-beige, yellow, yellowish-brown to ochraceous-yellow, ochraceous-orange to even white, and brown; when dry, beige to beige-orange, to light-brown, to dark brown, when dry 6–25 mm diam and 6–14 mm tall; when rehydrated 16–50 mm diam and 8–20 mm tall. **Stipe** terete or compressed, sometimes grooved, slender, hollow, slightly attenuate below and often forming a disc-like holdfast at the bottom, concolorous or even paler than the receptacle, 3–40(–75) x 1–6 mm. **Disc** deeply concave, appears smooth to the unaided eye, when fresh pink or salmon, or light-coral to coral to deep coral, orange, yellow, mauve or light-brown. **Margin** not inrolled at maturity, when dry inrolled, when fresh concolorous to receptacle, brown to light yellow when rehydrated, provided with several rows of long hairs. **Tomentum** of two types: 1) individual monilioid processes that are present on the margin and as well covering sparsely all the receptacle giving it a furfuraceous appearance, composed of 2–5 short hyaline cells, cells 20–30 µm diam with thick and rugose walls, walls 2–5µm thick. 2) fused triangular-shaped bundles of cells present at the margin, 40–100 µm long, 34–64 µm wide at the base, and 8–22 µm wide at the apex. **Hairs** located in 2-5 concentric ridges or rows around the margin, approximate distance between rows.µm wide, hyphae 2–3 µm wide, not staining with Congo Red. **Medullary excipulum** *textura porrecta* not 0.25–0.75(–1) mm; all hairs of approximate the same length, 0.25–1 mm long when dry, 0.75–1.25 mm long when rehydrated, each hair composed of fascicles of hyphae which are longitudinally fused, the exterior hyphae shorter than the others so the compound hair has a broad base up to 200 µm diam, diminishing in width up to a pointed apex, individual hyphae white to beige 4.5–9.0 µm diam. **Outer ectal exipulum** *textura globosa* to *angularis* or *prismatica*, layer (28–) 30–60 (–80) µm wide, composed by a few cell layers not staining with Congo Red, lying perpendicular to the surface of the receptacle, outer cells round to elongated, (15–)16–30 x (9–)12–24 µm, cells becoming rounder and thicker-walled towards the flanks, at the surface of the receptacle groups of thick-walled and sometimes warted, globose or pyriform cells are often irregularly aggregated causing the pruinose appearance of the receptacle; internal cells are smaller and prismatic. **Inner ectal excipulum** *textura intricata* to lax *intricata*, without a gel; this layer is gelatinized only in infertile young specimens, disappearing at maturity, layer (20–) 30–80 gelatinized, layer (30–) 40–300 µm wide, composed of long compressed parallel hyphae, 2–4 (–5) µm wide, not staining with Congo Red. **Subhymenium** 15–30 µm thick of *textura intricata* to *porrecta*, gelatinized in immature specimens, hyphae irregular, thread-like and loosely interwined with each other. **Hymenium** 200–390 µm tall, with interspread setae present. **Setae** sometimes dark, tortuose, thick-walled, exceeding the hymenium by 22–40 µm. **Asci** long cylindrical, (61.6–)250–430(–460) x 10–30(–40) µm, thick-walled, walls 2 µm thick; with 8 spores located in the top 2/3 on the ascus, round at the base and abruptly contracted into a basal hyphal appendix, appendix 6–24(–40) x 2–6(–10) µm. **Ascospores** uniseriate, ellipsoid to broad elliptic fusoid to subfusoid, somewhat asymmetrical, sometimes one end more pointed and the other rounder, with more or less projecting apiculi at the poles, subhyaline under the

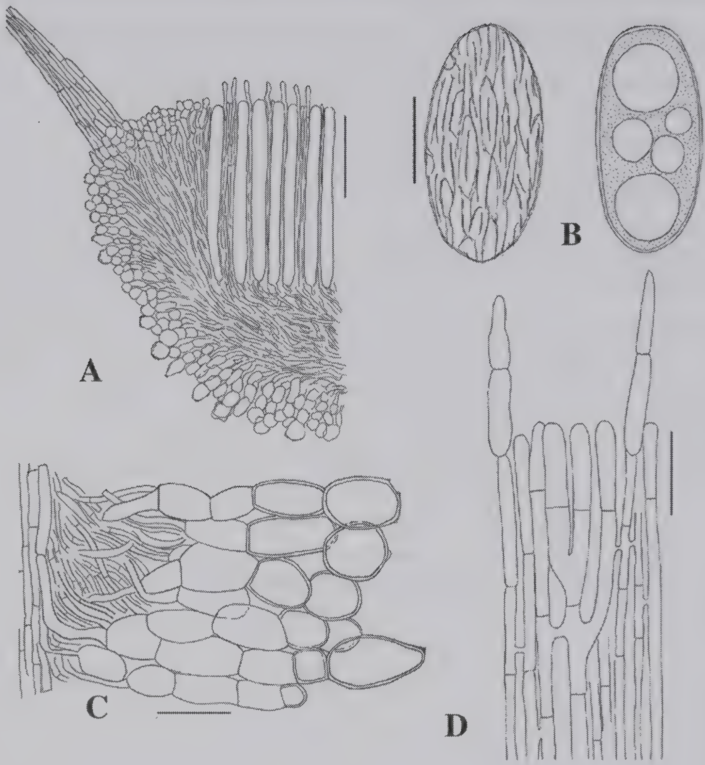


Figure 7. *Cookeina speciosa.* A. Cross section of an apothecium, bar = 120 µm. B. Ascospores, bar = 10 µm. C. Cross section showing outer layer of the excipulum, bar = 25 µm. D. Portion of hymenium with setose paraphyses, bar = 10 µm. Yutajé, Venezuela; *T. Iturriaga & K. Samuels*, 4A-D4; 4 Jul –7Jul 1997 (FH).

microscope, thick-walled, wall 1 µm thick, with 0–2(–multi) guttules, mostly with two large central guttules and numerous smaller oil drops, at maturity appearing striate from the presence of fine, rarely anastomosing paralled striate low ridges 1(–2) per µm, spores 20–36 x10–18(–20) µm. **Paraphyses** filiform, thread-like, delicate, simple or branching, anastomosing and forming a network of thin, septate, hyphae, sometimes with irregular peg–like and one–sided swellings at their middle, more profusely branched at their apex, ending in rounded clavate tips up to 3 µm diam, containing fine granules; same height as the asci, or exceeding them slightly, (1–)2–3 µm wide in the middle, 1–5(–7) µm wide at the apex.

SUBSTRATE: on wood, on fallen logs, twigs or branches.

DISTRIBUTION: Distributed throughout the tropics in both the new and the old world.

ILLUSTRATIONS: Plumier (1705) tab. 168, fig. C; Berkeley (1842) as *Peziza hindsii*, pl. 15; Berkeley (1852) as *P. hindsii*, tab. 15, fig. 4; Fries (1860) as *Peziza afzelii*, pl. 12, fig.

28; Cooke (1874) as *P. hindsii,* plate 27, fig 3; Cooke (1879) as *Peziza sulcipes* fig. 199, as *P. hindsii* fig. 200; Cooke (1892) as *Trichoschypha hindsii,* fig. 153; Hennings (1892) as *Pilocratera engleriana,* pl. VI, fig. 9 a, b; Seaver (1928, 1942) as *C. sulcipes,* pl. 18, fig.1; Le Gal (1953) as *C. sulcipes,* figs. 114–119; Rifai (1968) as *C. sulcipes,* figs 18–20; Nagao (1997) as *C. sulcipes,* fig. 1; Vooren & Lopez (2002) fig. 2; Melendez-Howell et al. (2003) as *C. sulcipes,* fig. 1, 3–5, 7.

NOTES : *Peziza speciosa* was based on Plumier's Tab. 168 Fig C, as pointed out by Dennis (1994). Prior to Dennis's observation this species was generally known under the name *C. sulcipes* and *C. hindsii.* The number of synonyms for this species is no doubt related to the diffuse mycological literature of the early to mid 19th century and also to the variability of this species in hymenial color. In an ITS sequence analysis all collections referred to *C. speciosa* fall within a monophyletic clade. Within that clade there is phylogenetic structure that indicates some genetic variation that correlates as well with color differences. It is perhaps best to consider this as a species complex until such time as more complete population studies are done. This and *Cookeina tricholoma* are the most common species of the genus in most parts of the tropics. The hairs in rows at the margin of the disc serve to distinguish this species from *C. tricholoma.*

For those who might wish to segregate color forms the following guide to the application of names might be followed: 1) *Pilocratera engleriana* is the oldest name for an orange–yellow species. It has not been combined in *Cookeina*. 2) *Peziza javanica* and *Peziza amoena* were both described in the same publication, one from Java and one from Guiana but both were described as yellow. 3) fawn or darker forms from Africa were referred to as *C. sulcipes* var. *fusca*. Our morphological studies did not find distinctions in size of asci, ascospores or apothecia to warrant recognition of these taxa.

GENERAL NOTES: In parts of Mexico *C. sulcipes* is used as a food as summarized by Villarreal & Pérez-Moreno (1989) and is listed among those fungi known and used by indigenous people in Mexico (Chacon 1988). It is used medicinally in Cameroon, Africa (Dijk et al. 2003).

SPECIMENS EXAMINED. AFRICA. CAMEROON: *Zenker* Nr. P41, from alcohol (B), Zenker, Aus der Alkoholsammlung, *P41* (B) [holotype of *Pilocratera engleriana*]; Mount Cameroon (IDENAU. lava floco) on leaf litter, coffee colour from transparencies inside packet, 1922, Alt (m): 2, *S. Dawson 45*, K (M): 57300. LIBERIA. CENTRAL PROVINCE: Gbanga, 20 Sept 1926, *D. H. Linder 726* (FH); 7 Sept 1926, *D. H. Linder 392* (FH); as above, 23 Sept 1926, *D. H. Linder 392* (FH); Kassa Ta, 29 Sept 1926, *D. H. Linder 392A* (FH); Mount Barclay, 20 Jul 1926, *D. H. Linder* (FH); Pehata, 8 Oct 1926, *D. H. Linder* (FH); UGANDA: Uganda Protectorate, decaying log. [400 Ft], Mt. Nayge, greyish ochre, abril 1916, *R. Dummer 2845* (K). BRAZIL. AMAZONAS: On rotten wood, June 1901, *2811* (FH). CHINA. YUNNAN: Monlin, Hsichuang Panna Autonomous Prefecture of Tai Nationality, on rotten wood, 17 Oct 1980, *Zang Mu 7240* (FH); Xishungbanna, Menlen, ex situ of endangered plants area, 500m alt, on rotten wood, 25 Oct 1988, *Zang Mu 11532,* 20381 (HKAS). COLOMBIA. MAGDALENA: San Sebastian, *Ruth Aldava 296* (FH). COSTA RICA. Cartago, Forest of Turrialba Instituto, 1700 ft, 17 Sept 1964, *W. C. Denison, et al. 2354, 2359, 2360, 2363, 2364* (=OSC 21542, 21547, 58562, 67758, 67760); as above, on stick, 19 Sept 1964, *Denison, et al. 2389* (= OSC 67770); as above,1700ft, 19 Sept 1964, *Denison, et al. 2394* (= OSC 67772); LIMÓN PROVINCE: Finca Castilla, 30m, 23, 25, 26, 29 Jul 1936, *C. W. Dodge & V. F. Goerger* 9314 (FH); Guapiles, Finca Diamante, on dead wood, 18 Sep 1964,. *Denison, et al. 2376*

(= OSC 67767); as above, 400ft, on dead wood, 18 Sept 1964, *W. C. Denison, et al. 2375, 2376, 2386* (= OSC 21516, 67766, 67768); as above, 700ft, dead stick, 18 Sept 1964, *Denison, et al. 2386* (= OSC 21548); Portete, 50ft, sticks, 26 Aug 1964, *Denison et al. 2111* (= OSC 67717); Westphalia, 10ft, 26 Aug 1964, *Denison et al. 2118* (= OSC 67719); PUNTARENAS: Coto, United Fruit Co., Finca 59, 75ft, on limb, 3 Oct 1964,. *Denison 2462* (= OSC 67784, 21531); as above, on rotting wood, 3 Oct 1964, *Denison 2453* (= OSC 67781, 21549); as above, 75ft, dead wood, 3 Oct 1964, *Denison 2452* (= OSC 67780); as above, 75ft, 3 Oct 1964, *Denison, et al. 2454* (= OSC 67782); Golfito, Ravine back of Balneario, rotting wood, 2 Oct 1964, *W. C. Denison 2448, 2449* (= OSC 21512, 21550, 67777, 67778); Above Golfito, 150ft, on rotten wood, 29 Aug 1964, *Denison et al. 2158* (= OSC 67720, 21530); Gorge back of Golfito, 150ft, dead wood, 30 Aug 1964, *Denison, et al. 2162* (= OSC67723, 21544); Hills above Golfito, 200ft, stick, 30 Aug 1964, *Denison, et al. 2179, 2181* (= OSC 67726, 67727); Guanacaste, Tilaran, 1700ft, rotten limb, 15 Sept 1964,. *Denison, et al. 2330* (= OSC 67753, 21517); as above, Rotting wood, 15 Sept 1964, *Denison et al. 2329* (= OSC 67752); Alajuela, hot springs near La Marina, 1400ft, branch on ground, 11 Sept 1964, *Denison et al..2262* (= OSC 67742); as above, 1500ft, rotting wood, 11 Sept 1964, *Denison et al. 2257, 2258* (= OSC 21518, 67739); Palmar Norte, 200ft, 30 Aug 1964, *Denison, et al. 2186* (= OSC 67729. 21532); Gorge just north of Palmar Norte, 175ft, 31 Aug 1964, *Denison, et al. 2195* (= OSC 67731, 21515); as above, 175ft, stick, 31 Aug 1964, *Denison, et al. 2197* (= OSC 67732); PanAm Highway north of Palmar Norte, 200ft, on stick in cacao plantation, 1 Oct 1964, *Denison 2444* (= OSC 67774); San Jose, University of Costa Rica, 3500ft, standing dead tree, 25 Aug 1964, *W. Westman, Denison 2109* (=OSC 67716, 21521). CUBA. 1856–7, *C. Wright* (FH); *C. Wright* (FH). DOMINICAN REPUBLIC. SEIBO: Canada Hondo, on dead sticks, Nov 1946, *R. A. & E. S. Howard* (FH); Higuey, on fallen logs in dense woods, Nov 1946, *R. A. & E. S. Howard* (FH). FRENCH GUIANA: Vicinity of Cayenne, taken from material in 418, 1921, *W. E. Broadway 418b* (FH). GUATEMALA. 'Alsacia' Mountains, 1200ft, in wet ravine, 17 Jul 1936, *Winslow Hatch 411* (FH). GUYANA. Plantation Vryheid, 2 Feb 1924, *D. H. Linder 843* (FH); Bartica, 9 Dec 1923, *D. H. Linder 464* (FH). JAMAICA. Portland, Blue Mountains, Stony River Base Camp, 1250ft, in woodland, on scree, on fallen branch, 13 Dec 1973, *B. D. Morley & C. Whitefoord 505* (FH); Jul 1902 (FH); Port Antonio, Road to Park Mount, 6 Feb 1906, *A. E. Wight 218* (FH); St. Margaret's Bay, 26 Feb 1906, *A. E. Wight* (FH). PANAMA. Barro Colorado Island (FH); On spring palm stem, 12 Dec 1928, *W. H. Weston 3298* (FH); Dec 1928 (FH); Canal Zone, trail below Madden Dam, 250ft, on branch, 7 Oct 1964, *Denison 2465, 2466* (=OSC 67787, 67788, 67792, 21541); Las Cruces Trail, 400ft, on rotting wood, 7 Oct 1964, *Denison 2471, 2474* (= OSC 67790, 67792). PAPUA NEW GUINEA: Kumusi River, 1859, *W. Fitzgerald 58274* (K); New Ireland, *R. B. N. 58275* (K). PHILIPPINES. ISABELA PROVINCE: Luzon Island, Planan, Sitio Dipaguiden, Branagay San Isidro, on decayed log in shade, 16 Apr 1991, *Benito C. Tan 361* (FH); Laguna, Los Baños, on dead wood, 24 Oct 1921, *C. G. Wells 11670* (NY); Island of Luzon, Los Baños (Mt. Maquiling), Province of Laguna, June-July 1917, *18329* (FH); On dead wood, 24 Oct 1921, *Colin G. Welles* (CUP 11683); University of the Philippines Campus, College of Agriculture, on dead wood, Dec 1958, *Lewis A. Schafer* (NY); Mud Spring, on wood, 24 Nov 1966, *Denison 3938* (= OSC 24423); as above, Dead branches, 27 Nov 1966, *Denison 4730* (= OSC 28876); as above, Wood, 24 Nov 1966, Rebeca de Guzman, *Denison 3943* (= OSC 24418); as above, On dead logs, 23 Aug 1966, *Denison 4706* (= OSC 27971); Quarry, in bark of dead wood, 13 Oct 1966, *Denison 3940* (= OSC 24421). SURINAM. Tafelberg, 390m, 11 Aug 1944, *Bassett Maguire* 24312 (FH). TRINIDAD. Arima, Verdant Vale, 1913, *R. Thaxter* (FH); Arima Valley, Jan 1975, *Sylvia Stein* (NY); 1879, *Rev. M. J. Berkeley 35303* (K); Maravel Valley, on wet decaying wood at border of brook, 18 Aug 1923, *D. H. Linder 66* (FH); Port of Spain, 1912–13, *R. Thaxter 3698* (FH); 1913, *R. Thaxter 3699* (FH). VENEZUELA. AMAZONAS: Oeste del Caño Yutajé, 1km NE del Campamento Yutajé, Bosque de las Ceibas, pie de monte, 5°35'N, 66°10'O, tronco muerto, 21 Jun 1996, *Teresa Iturriaga y colaboradores 2721, 2722, 2723* (USB); NE del Campamento Yutajé, Bosque en el lado norte del río Yutajé, 5°36'51"N, 66°6'85"W, madera muerta, 15 Jun 1996, *Teresa Iturriaga y colaboradores 2596* (USB); Yutajé, Bosque primario donde está localizada la parcela 'FEX,' madera en descomposición, 17 Jun 1996, *Teresa Iturriaga y colaboradores 2610, 2612, 2613* (USB); Yutajé, 80 collections by *K. Samuels* as part of an ecological study, 4 Jul –7Jul 1997 (FH, USB); BOLIVAR: Sifontes, Tumeremo, Carretera Tumeremo – Bochinche, Campamento maderero de INTECMACA, orillas del Río Botaramo, sobre tronco, 17–19 Nov 1994, *T. Iturriaga, L. Bracamonte, L. Ryvarden, O. Holmquist 2167, 2170, 2180, 2185* (USB); MIRANDA: Parque Nacional Guatopo, 10°03'N, 66°26'W, 500–600m, on decorticated wood, 27–30 Nov 1990, *G. J. Samuels, B. Hein, S. M. Huhndorf* 7680 (FH); *G. J. Samuels, B. Hein, S. M. Huhndorf 7681* (FH).

Cookeina tricholoma (Mont.) Kuntze, Revis. Gen. Pl. 2: 849. 1891. **Figs. 1 A, B, 2**

≡ *Peziza* (*Lachnea*) *tricholoma* Mont., Ann. Sci. Nat. Bot. II 2: 77. 1834 !! ≡ *Lachnea tricholoma* (Mont.) Pat., Bull. Soc. Mycol. France 4: 98. 1888. ≡ *Trichoscypha tricholoma* (Mont.) Sacc., Syll. fung. 8: 160. 1889. ≡ *Pilocratera tricholoma* (Mont.) Henn., Bot. Jahrb. Syst. 17: 9. 1891.

= *Peziza tricholoma* [var.] ß *minor* Mont., Ann. Sci. Nat. Bot. II 2: 77. 1834.

= *Peziza hystrix* Berk. Ann. Mag. Nat. Hist. II 9: 201. 1852 !!

= *Pilocratera tricholoma* var. *celebica* Henn., Monsunia 1: 33. 1900.

= *Peziza medusina* Speg. Anales Mus. Nac. Hist. Nat. Buenos Aires III 1: 78. 1902 !! ≡ *Pilocratera medusina* (Speg.) Sacc. & D. Sacc., Syll. fung. 18: 32. 1906 !!

= *Peziza (Sarcoscypha) striispora* Ellis & Everh. Bull. Lab. Nat. Hist. Iowa State Univ. 4: 69. 1896. ≡ *Sarcoscypha striispora* (Ellis & Everh.) Sacc., Syll. fung. 14: 754. 1899.

Apothecia solitary or gregarious, deep cupulate, centrally stipitated to rarely subsessile, frequently elongated on one side being then suboblique, leathery, receptacle and stipe covered with long conspicuous hairs more abundant at margin specially in young apothecia, when dry 7–25 mm tall and (5–)10 x 35(–50) mm diam, when rehydrated 20–25 mm tall. **Receptacle** when fresh 10–40 mm diam and 5–15 mm deep, paler than the disc, orange to yellowish-orange, to reddish, to pinkish, to coral red; when drying 5–20 mm diam and light reddish-brown to beige, when rehydrated 10–35(–50) mm diam, orange to yellow-ochraceus to yellowish brown. **Stipe** central or slightly eccentric, slender, concolorous with receptacle, when fresh fleshy, white, orange-buff to salmon-orange to beige, when dry tough, 2–35(–50) x 1–4 mm, cylindrical or compressed, with hairs mainly present on the upper half, these being shorter than the ones in the receptacle. **Disc** smooth, variable in color, concolorous with receptacle or lighter, when fresh pale orange, to buff-orange to rose-orange, in old specimens nearly whitish, when rehydrated light yellow. **Margin** enrolled in dry specimens, covered with abundant long conspicuous hairs especially in young apothecia and by short hairs (tomentum). In young apothecia the hairs form an in-curved border that close the apothecium. **Hairs** whitish (especially in younger specimens) to brownish-yellow to brown, when dry cream-colored to beige, fasciculate, darker at the base, covering all margin and receptacle, composed of septate parallel hyphae 2–4 µm wide, arising from the inner ectal excipulum and breaking though the outer ectal excipulum at a right angle. Marginal hairs up to 7 mm long and up to 250 µm diam, twice as wide as the receptacle hairs, abundant, 2 per mm. Receptacle hairs located on the upper 1/3 of the receptacle or covering it completely, 2–4 mm long and 64–90 µm diam in the middle of the fascicles, widening up to 90–130 µm diam at the base. Hairs originate from the medullary excipulum and penetrate the ectal excipulum extending obviously above the receptacle's surface. **Tomentum**, of two types: 1) individual monilioid processes that arise from the margin and also sparsely cover the receptacle, these cells produce the pruinose appearance of the receptacle, these catenulate processes composed of 3–5 short hyaline cells, cells 10–20 µm diam, outermost cells with thick and rugose brownish thick walls, hairs 40–80 x 7–10 µm. 2) fused triangular-shaped bundles of the catenulate (monilioid) cells present at the margin. **Outer ectal excipulum** of a few layers of cells of *textura globulosa* to *angularis*, 10–72(–100) µm wide, cells arranged perpendicularly to the surface of the receptacle, cells 7–25 x (5.5–) 7–18(–20) µm. Outer cells with thick walls which are roughened become loose and grouped, giving the receptacle a pruinose appearance. **Inner ectal excipulum** of loose *textura intricata* of thin-walled hyphae oriented perpendicular to the receptacle's surface, layer 30–60 µm wide, hyphae 2–4 µm wide, no gel present. This layer is almost non-existent in wholly mature specimens, but can be evident in young specimens, where some gelatinization of this layer may be present as well. **Medullary excipulum** *textura porrecta* without gel of 160–200 µm wide, parallel or seldom branching septate hyphae composed of long cells with some spaces occasionally between them, hyphae of 2–7.5 µm diam. **Subhymenium** loose *textura intricata*, layer 40–60 µm wide, hyphae up to 5–6 µm diam. **Hymenium** 260–440 µm thick. **Asci** cylindrical, with rounded blunt base, 255–390 x 10–30 µm, walls up to 2 µm wide, connecting to short, narrow basal hyphae, 6–14(–30) x (1–) 4–6 µm, with 8 ascospores. **Ascospores** uniseriate, elliptic-fusoid, apiculate at both ends though one end frequently is more pointed than the other, subhyaline, (0–) 2 large guttules and several smaller ones may be present as well; with an inner and outer wall, at maturity distinct markings present, consisting of fine parallel longitudinal, low, ridges which sometimes anastomose 1–2 per µm, wall 1 µm diam, 25–39 x 12–21 µm, normally restricted to the top 160–190 µm of the ascus. The ornamentation is only seen in mature spores. **Paraphyses** filiform, septate, hyaline, branched and frequent anastomosiing to form a dense network, especially very profusely near the apex where they form short erect branches and end in a compact layer, 1.5–4.5 µm in the middle, enlarging at the apex in a clavate shape to 2–6 µm, exceeding the asci.

SUBSTRATE: On wood, on fallen logs, twigs or branches.

DISTRIBUTION: Throughout the lowland tropics, both new and old world. The species has on occasion been collected in Florida.

ILLUSTRATIONS: Montagne (1834) as *Peziza tricholoma*, Plate 4, fig. 2; Cooke (1874) as *P. tricholoma,* pl. 27, fig. 4, as *P. hystrix* pl. 27, fig. 12; Cooke (1879) as *Peziza tricholoma* f. 202; Ferdinandsen & Winge (1910) fig. 4, page 218; Seaver (1913) fig. 1, 2; Seaver (1928, 1942) pl. 18, fig. 2; Boedijn (1933) as *C. tricholoma*, fig. 2b, 3h, 4a, 4e; Le Gal (1953) fig. 105–108; Dennis (1954) as *C. tricholoma*, fig. C; Gamundí (1959), fig. 1–8; Denison (1967) fig. 3, 5-6; Rifai (1968) fig. 15–17; Dennis (1970) fig. 7U; Otani (1971) plate 3 c, fig. 15, 16; Gamundí (1983) map 6, distribution map for neotropics; Weinstein et al. (2002) fig. 3D; Vooren & Lopez (2002) fig. A; Melendez-Howell et al. (2003) fig 3c, 7e– g, 14.

NOTES ON TYPE SPECIMENS AND NOMENCLATURE: Montagne's description of *P. tricholoma* is based on material from Brazil from the herbarium of Gaudichaud collected in 1831/1832 around Rio de Janeiro and labeled no. 44. There are three collections at PC that fit this information. All match the original description and all are labeled number 44. After examining the three collections we have selected one as lectotype because of its good state of preservation and maturity.

Peziza tricholoma var. *minor* was described at the same time as *P. tricholoma* by Montagne (1834), he mentioned that this variety was different from *P. tricholoma* because it is almost glabrous with few setae "setis raris vel nullis" and that it is smaller. From Montagne's description, it is evident that ß minor was collected in or at the vicinity of Rio de Janeiro, Brazil. There is one collection in Montagne's herbarium that does not bear a collection number, collected by Gaudich, and it is from Rio de Janeiro. **We designate this collection as the lectotype of var. *minor*.**

Peziza hystrix was erected by Berkeley (1852) in the belief that *P. tricholoma* was a smaller species, that the external surface was costate, and that the hairs in P. *tricholoma* covered only the margin as shown in Montagne (1834) figure 2a (see list of illustrations below). Montagne did mention that there were some hairs on the external surface, but that these were mainly present, and in a higher number, at the margin. Cooke (1879) examined Montagne's specimen and found that the hairs covered the receptacle and stipe. Having seen both Montagne's and Berkeley's collections, Cooke concluding that *P. hystrix* was a synonym of *P. tricholoma*.

Peziza (Pilocratera) medusina was considered to have smooth ascospores (Spegazzini 1902). We examined the type specimen of *P. medusina* and have observed striations present on the ascospores. We conclude that this is a synonym of *C. tricholoma*, as Gamundí (1959) had already stated.

GENERAL NOTES: *Cookeina tricholoma* and *C. speciosa* are the commonest species of *Cookeina* encountered. In parts of Mexico *C. tricholoma* is used as a food as summarized by Villarreal & Pérez-Moreno (1989) and is listed among those fungi know and used by indigenous people in Mexico (Chacon 1988). It is used medicinally in Cameroon, Africa (Dijk et al. 2003). *C. tricholoma* can be easily distinguished by the more or less uniform covering of hairs on the outer surface of the apothecium. Boedjin (1933) indicated that ascospores are faintly rose in color, and that spore deposits of this species were pink. Spore prints are not routinely made of discomycetes and so we do not have comparative data on spore colors in other species. Alasoadura (1972) indicates that the hairs are covering the "mouth" of the apothecium at night and early morning, that is, when spore discharge is not appreciable, at other times the hairs point upwards leaving the "mouth" of the cup open. Boedijn (1933) indicates that the ascospore has a thick inner and a thinner outer wall; he observed that for a long time the outer spore wall remained smooth, but when fully ripe, the outer wall shows a delicate longitudinal striation, caused by low, sparingly anastomosing ridges. Melendez-Howell et al. (2003) have made a comprehensive study of wall layers in asci, paraphyses and ascospores, as well as SEM ascospore observations. They show that ascus walls in *C. tricholoma* are thick; that there is a gel layer that covers the ascus, thinning out towards the apex, that there are multiple randomly located germ pores on ascospores of *C. tricholoma*, and that the low ridges that form the ascospore ornamentation have a slightly spinose surface (see list of photographs below). They state that ascospores may be covered by the perispore, which masks somewhat the ornamentation of the "paroí propre."

Specimens examined. AFRICA. CAMEROON: Sakbayene, 1926, *Rev. Charles Schwab* (FH); June 1918, *28* (FH-Pat); Sur un bronc pourrissant d'un Eryshrina caralledenstrae, *1483* (FH-Pat); June 1918, *21* (FH-Pat); June 1918, *19* (FH-Pat). CONGO: Nov 1893-Mar 1894, *Dybowski* (FH-Pat); LIBERIA: Gbanga, 20 Sept 1926, *D. H. Linder 727* (FH); Sept 1926, *D. H. Linder 392* (FH). ARGENTINA. S/ramas muertas, Misiones, Puerto Pampa, 8-4-1901, Leg. E. Kermes [holotype of *Peziza medusina*] (Packet from LPS bears the number 28026) BRAZIL: Ad ligna, Rio de Janeiro, Brazil, Gaudich. no 44 (PC), [lectotype of *Peziza* (*Lachnea*) *tricholoma*, designated by Iturriaga & Pfister] (PC); Rio de Janeiro, 1831-33, *44*, [isolectotype of *Peziza* (*Lachnea*) *tricholoma*, designated by Iturriaga & Pfister] (PC); 1831-33, [isolectotype of *Peziza* (*Lachnea*) *tricholoma*, designated by Iturriaga & Pfister] (PC); ad ligno, Rio de Janeiro, Exdono Gaudichii, (as *Peziza tricholoma*) [lectotype of *Peziza tricholoma* var. ß *minor*, designated by Iturriaga & Pfister], Rio de Janeiro (PC), Crypt. Guyani 444, sur le petioles pourris de l'...carium vulgare, *890* (PC); Crypt. Guyani 444, *891* (PC); Guyani (PC); Amazonas, Mowary, Juruá, auf vermodertom Hok, Sept 1900, 2812 (FH); Porto Novo, Sta. Catharina, 1978 (FH); on rotting log, bank of brook in shady gully, 5 Jan 1906, *A. E. Wight 137* (FH); St. Catharina, mar 1888 (FH). CHICALAPA. Sur le bois pourri dans les grandes forêts, ils sous eouges ou violes et en forme de vase, Dec 1857, *Sallé 303* (FH-Pat); JAVA: Iter javanicum secundum, *H. Zollinger 2042* (FH-Pat); Ile Sansos Hebrides, Jul 1906, *Le Rat 12* (FH-Pat). CHINA. KWANGSI PROVINCE: Ta Chai Shan, 1933, *S. Y. Cheo 2391* (FH); YUNNAN: Hsichuang Panna, Monlin, Calcareous Mountain, on rotten twigs, 3 Sept 1974, *Zang Mu 1171* (FH); Mengla, on rotten wood, *Zhuliang Yang 23238* ex HMAS (FH); On wood, 1 Nov 1999, ex HMAS (FH). COSTA RICA. LIMON: Portete, 50ft, on stick, 26 Aug 1964, *Denison et al. 2112* (= OSC 67718); Guapiles, Finca Diamante, 700ft, stick, 18 Sept 1964, *Denison et al. 2387* (= OSC 67769); PUNTARENAS: Pan-American Highway South of Buenos Aires, 430ft, in a ravine with waterfall, 29 Aug 1964, *Denison et al. 2160* (= OSC 67722); San Jose, 15 km. South of San Isidro del Gen., 1600ft, stick, 29 Aug 1964, *Denison et al. 2159* (= OSC 67721); Guanacaste, El Silencio, 2500ft, on large limbs, 15 Sept 1964, *Denison et al. 2319* (= OSC 67751); San Vito, Oxan's, 4000ft, on sticks, 22 Oct 1964, *W. C. Denison 2536, 2537* (= OSC 67794, 67795); Cartago, Turrialba, forest at Instituto, 1700ft, on wood, 17 Sept 1964, *Denison et al. 2351* (= OSC 67754); as above, Dead wood, 17 Sept 1964, *Denison et al. 2352, 2357* (= OSC 67755, 67757); Guanacaste, north of Puntarenas, 100ft, wood, 13 Sept 1964, *Denison et al. 2285* (= OSC 67745); PanAm Highway, 100ft, on rotten wood, 13 Sept 1964, *Denison et al. 2284* (= OSC 67744); near Santa Cruz, 100ft, wood, 14 Sept 1964, *Denison et al. 2295* (= OSC 67747); as above, Sticks, 14 Sept 1964, *Denison et al. 2316* (= OSC 67750); Santa Cruz, 100ft, on rotten limbs, 14 Sept 1964, *Denison et al. 2315* (= OSC 67749); Caña, 150ft, 13 Sept 1964, *Denison et al. 2288* (= OSC 67746); Rio Pobrenos, 350ft, 13 Sept 1964, *Denison et al. 2306* (= OSC 67748); Alajuela, La Marina, Hot Springs, 1500ft, 11 Sept 1964, *Denison, Jiménez et al. 2256, 2261, 2264* (= OSC 67738, 67741, 67743); as above, On stick, 11 Sept 1964, *Denison, Jiménez et al. 2259* (= OSC 67740); as above, 1500ft, rotten wood, 10 Sept 1964, *Denison et al. 2252* (= OSC 67736); as above, On dead branch, 11 Sept 1964, *Denison et al. 2255* (= OSC 67737); Alajuela, Buena Vista, Finca Ensayña, 2800ft, 10 Sept 1964, *Denison et al. 2246* (= OSC 67734); as above, On rotten wood, 10 Sept 1964, *Denison, Jiménez, et al. 2247* (= OSC 67735); Gorge near Golfito, 150ft, on sticks, 30 Aug 1964, *Denison et al. 2163* (= OSC 67724); as above, 200ft, 30 Aug 1964, *Denison et al. 2183* (= OSC 67728); as above, 30 Aug 1964, *Denison et al. 2168* (= OSC 67725); Golfito, near Hotel Balneario, 50ft, on rotten wood, 2 Oct 1964, *Denison 2446* (= OSC 67775); Ravine back of Balneario, 50ft, dead wood, 2 Oct 1964, *Denison 2447* (= OSC 67776); Coto, United Fruit Co., Finca 59, 75ft, wood, 3 Oct 1964, *Denison 2451, 2455* (= OSC 67779, 67783); Palmar Norte, 175ft, 31 Aug 1964, *Denison et al. 2192* (= OSC 67730). CUBA. Trinidad Mountains, Sierra de San Juan, Mina Carlota, on log, 5 Jul 1941, *W. L. White* 722 (FH); Amazon Basin, 1000 ft, on old rotten log in forest, 19 Jan 1922, *O. E. White 2358* (FH); *C. Wright 664* (FH); C. *Wright 665* (FH); *C. Wright* (FH-Pat); *C. Wright 657* (FH); *C. Wright* (FH); SANTA CLARA: Soledad, La Veguita de San Antonio, 17-23 Aug 1935, *D. H. Linder* 72 (FH). DOMINICAN REPUBLIC. On dead wood, Salle, no. 35, St. Domingo [holotype of *Peziza hystrix*] (K); Seibo, on humus in deep woods, Nov 1946, *R. A. & E. S. Howard* (FH). FRENCH GUIANA. Vicinity of Cayenne, on ground beneath trees, 6 Jul 1921, *W. E. Broadway, 692* (FH). GUADELOUPE. Capesterre, 100-250 m, on twig in banana plantation, 10 Jan 1974, *D. H. Pfister, S. Carpenter, M. Sherwood 1190* (FH); Pointe-Noire, sur les peliles branches pourries, *61* (FH-Pat); Sur un morcean bois pourri d'un Cheobroma Cacao, *Duss 980* (FH-Pat); Sur un branc pourri de Megrisleca moscati, *Duss 998* (FH-Pat); Sur un éclas de bois pourrissant, 1902, *Duss 623* (FH-Pat). GUATEMALA. Alta Verapaz, on decaying fallen log, 27 Jul 1936, *Winslow Hatch 413* (FH); Los amates, 'Alsacia' Mountains, on decaying wood, 17 Jul 1936, *Winslow Hatch 412* (FH). GUINÉE FRANCAISE. Simbaïa, 20 Apr 1909, *Duport* (FH-Pat); St. Domingo, *58273* (K). GUYANA. Bartica, Dec 1923, *D. H. Linder* (FH). INDIA. KERALA: Wynad, Periya Reserve Forest, on fallen twigs on forest floor, 23 Aug 1984, *P. Manimohan* (FH). INDONESIA. JAVA: *Tjiboya 2042* (FH-Pat) [as *Peziza aurantia* var. *stipitata*]; Iter javanicum secundum, *H. Zollinger 2039* (FH-Pat). JAMAICA. 1909, *A. E. Wight* (FH). MEXICO. Circa le Alagirines, S.L.P. 11 Aug 1891, *7084* (FH-Pat). VERACRUZ. Sur des branches de bois mort á terre dans la forêt, Oct 1854, *Sallé 50* (FH-Pat). OUBANGUI. 1891, *Dybowski* (FH-Pat); Bois mort, 1891, *Dybowski* (FH-Pat). PANAMA. Canal Zone, on dead log, 8 Oct 1946, *N. L. H. Krauss 76* (FH); Canal Zone, Las Cruces Trail, 7 Oct 1964, *Denison 2475* (= OSC 67793); as above, Dead wood, 400ft, 7 Oct 1964, *Denison 2473* (= OSC 67791); Alahuela, Madden Dam, Azote Caballo Road, 90-100m, 27 Nov 1934, *C. W. Dodge 8952* (FH); Trail below Madden Dam, 250ft, stick, 7 Oct 1964, *Denison 2463*, 2464 (= OSC 67785, 67786); Madden Dam, 250ft, 7 Oct 1964, *W. C. Denison 2467* (= OSC67789); Sabanas near Chepo, 30m, 20 Jan 1935, *A. A. Hunter & P. H. Allen 8584* (FH); Barro Colorado Island, *Seephot 3385* (FH);. PHILIPPINES. RIZAI: Luzon, Aug 1913, *M. Ramos 21945* (FH); Luzon, Mt. Maguiling, on dead wood, 4 Oct 1920, *Medina 10291* (FH-Pat); 26 Oct 1920, *R. Reyes 10408* (FH-Pat); 25 Oct 1920, *P. Malabassan 10444* (FH-Pat); 23 Sept 1917, *S. Marquez 3393* (FH-Pat); Oct 1920, *O. A. Reinking 10269* (FH-Pat); 24 Oct 1920, *F. Bernardo 10446* (FH-Pat); 20 Oct 1920, *A. Abesamis 10417* (FH-Pat); 29 Aug 1917, *R. Reyes 3324* (FH-Pat); 30 Aug 1917, *S. Marquez 3393* (FH-Pat); 25 Oct 1920, *P. Malabassan 10459* (FH-Pat); 11 Oct 1920, *P. Lisou 10295* (FH-Pat); Mud Spring, along trail, 27 Aug 1966,. *Denison 4728* (= OSC 28874); Mt. Mekiling, Decaying bark of palms, 8 Oct 1966, B. D. Ona, *Denison 3946* (= OSC 67797). PUERTO RICO. Rio Sabana, 65°43'30"W 18°21'N, on partially buried twigs, 17 Jan 1996, *D. H. Pfister, F. A. Harrington, D. J. Lodge* (FH); On decaying wood, 17 Jan 1996, *D. H. Pfister & F. A. Harrington* (FH); On decaying wood, 17 Jan 1996, *D. H. Pfister & F. A. Harrington* (FH); Rio Pedras, 19 Dec 1911, *J. R. Johnston 173* (FH). TRINIDAD. Port of Spain, on partly submerged wood, 8 Aug 1923, *D. H. Linder 32* (FH); Port of Spain, 1912-1913, *R. Thaxter 3734* (FH); Port of Spain, Maraval, 1912, *R. Thaxter* (FH); St. Anne's Valley, on dead wood, 22 Aug 1923, *D. H. Linder 106* (FH); *R. Thaxter* (FH); 1913, *R. Thaxter 3802* (FH). USA. FLORIDA: Dade

County, Castellow County Park, on sticks, 10 Oct 1997, *J. Trappe 19953* (= OSC 60205). VENEZUELA. AMAZONAS: Yutajé, 5–7 Jul 1997, *K. Samuels,* 18 collections in USB; Oeste del Caño Yutajé, 1km NE del Campamento Yutajé, Bosque de las Ceibas, pie de monte, 5°35'N, 66°10'O, rama Muerta, 21 Jun 1996, *T. Iturriaga y colaboradores 2705* (USB); Madera en descomposición, 21 Jun 1996, *T. Iturriaga y colaboradores 2738* (USB); 21 Jun 1996, *T. Iturriaga y colaboradores 2738* (USB); 21 Jun 1996, *T. Iturriaga y colaboradores 2705* (USB); SOLANO: San Carlos trail, 100-130 m, on dead branches, 1959, *J. J. Wurdack & L. S. Adderley 43401* (FH); Tobagan de la Selva, Caño Coromoto, 75m, on a rotten trunk, 17 Oct 1988, *G. A. Romero 1772* (FH); Entre Maypures et San Fernando, sur branche pourrie, Aug 1887, *242* (FH-Pat); Entre Maypures et San Fernando, 27 Aug 1887, *Gaillard 243* (FH-Pat).

Cookeina venezuelae (Berk. & M.A. Curtis ex Cooke) Le Gal, Prodr. Flore Madagascar 4: 241. 1953. **Figs. 1 F, 2, 8**

≡ *Peziza venezuelae* Berk. & M.A. Curtis ex Cooke, Mycographia p. 120. 1875! ≡ *Phillipsia venezuelae* (Berk. & M. A. Curtis ex Cooke) Massee, J. Linn. Soc. 31: 473. 1896.
= *Discina pululahuana* Pat., Bull. Soc. Mycol. France 9: 145. 1893!
= *Discina epixyla* Pat. in Duss, Énumération méthodique des champignons recueillis à la Guadeloupe et à la Martinique p. 63. 1903!, !! (paratype)

Apothecia solitary to clustered, slightly to deeply cupulate, sessile to subsessile, when fresh up to 30 mm diam, when dry up to 20 mm diam. **Receptacle** concolorous or slightly lighter than the disc, frequently drying in a venose, convoluted-cerebriform ribbed pattern, more evident at the base of the receptacle where it shows a ribbed or venose pattern; apparently smooth but when closely examined minutely pubescent, and under the dissecting scope one observes that it is covered by a fine tomentum. **Stipe** present or lacking (sessile), when present central or slightly excentric, obconic, very short, 1–3 x 2 mm, concolorous to receptacle. **Disc** glabrous; when fresh, salmon to rose pinkish, when dry, cream colored to beige to light yellowish to ochraceous to light brown. **Margin** with 1–4 low circumferential ridges, which become more evident when dry, when rehydrated only uppermost ridge remains evident; under magnification appearing downy, and under the compound microscope there are evident short bundles, each bundle 30–120 µm long by 20–50 µm wide at the base, by 10–20 µm wide at the apex, each composed of several monilioid processes that cluster together; each monilioid process is formed by 4–5 round to ellipsoid cells in a filament, terminal cell of the filament round or tapering to a blunt point, cell walls thick and rugose due to encrustation or deposition, cells 60–70 x 10 µm. These bundles are more evident on the uppermost ridge of the margin. **Outer ectal** of the following: a) occasional groups of 5–12 thick-walled globose or elongate cells which extend to form pustules b) 2-celled short hairlike processes with globose, clavate or pyriform terminal cells c) monilioid filamentose hairlike processes composed of 3–5 round to ellipsoid cells; the two last ones (b & c) extend to form short hairs or "hairlike processes" as seen on the receptacle surface under a hand lens or dissecting scope 2) Inner ectal excipulum gelatinized, 66–88 µm thick, hyphae 1.5–4 µm in diameter, parallel to one another, but perpendicular to the outside of the apothecium and surrounded by refractive gelatinous material, which does not stain in cotton-blue lactophenol. **Medullary excipulum** 80–90 µm thick of *textura porrecta* with hyphae parallel to one another and also parallel to the outer surface of the apothecium, 3.0–

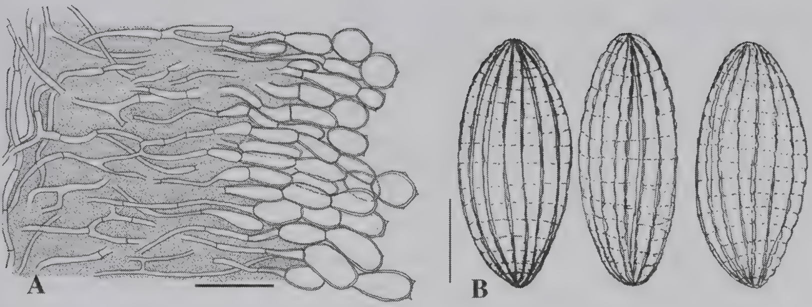


Figure 8. *Cookeina venezuelae*. A. Cross section showing outer layer of the excipulum, bar = 50 µm. B. Schematic representation of ascospores with longitudinal and transverse markings, bar = 10 µm. Haut Matouba, Guadeloupe, FWI; 9 Jan 1974, *D. H. Pfister, S. Carpenter, M. Sherwood 1161* (FH).

3.5 µm in diam. **Subhymenium** *textura intricata* to *porrecta*, not a well defined layer. **Asci** cylindrical, base round to slightly tapering, 275–550 x (10–) 20–30 µm, abruptly rising from thin basal hyphae (8–)12–32(–38) x 2–6 µm, 8 spores located at top 1/2 of the ascus. **Ascospores** elliptic fusoid, apiculate at each end, bilaterally symmetrical or asymmetrical, pale yellow, (24–)30–43 x (9.0–)14–18 µm, ornamentation consisting of few longitudinal ribs separated 3–4 µm from each other, and these connected by many fine transverse interconnecting ridges, (0–)2(–3) large central guttules surrounded by smaller ones towards both ends, uniseriate. **Paraphyses** filiform, septate, simple to branching, highly anastomosing, forming an interconnecting network, 2–5 µm diam in the middle, sometimes slightly enlarging at apex to 5 µm diam. **Excipulum** of *textura globulosa* to *angularis*, 3–5 cells thick, cells 6–20(–24) µm diam, radially arranged, outer layer of cells giving rise to one or several

SUBSTRATE: On decorticated wood, on wood and branches.

DISTRIBUTION: Known from Central America, northern South America (Venezuela is the type locality of *P. venezuelae*), Jamaica, Puerto Rico and Guadeloupe (type locality of *D. epixyla*).

ILLUSTRATIONS: Cooke (1875) pl. 69, fig 305; Cooke (1879) tab. 69, fig. 305. Cooke (1879) as *Peziza venezuelae* f.; Massee (1896) as *Phillipsia venezuelae*, tab. 16, fig. 4–5; Seaver (1928, 1942) as *Cookeina colensoi*, pl. 17, fig. 3; Le Gal (1953) fig. 109; Dennis (1954), fig. G; Gamundí (1957), Lam. II; Denison (1967) fig. 2, 4; Dennis (1970), fig. 7W; Pfister (1974), Fig. 1; fig. 9-11; Gamundí (1983) map 6, distribution in neotropics; Weinstein et al. (2002), fig. 3C.

NOTES ON TYPE SPECIMENS AND NOMENCLATURE: Some apothecia of the holotype of *Peziza venezuelae* (K) are sterile, but at least one of the apothecia is fertile, in good condition and many ascospores were seen by Le Gal (1953) and by us. Several parts of Fendler's original collection exist. One in the Sprague Herbarium (FH) is immature, a collection in the Ellis Herbarium (NY) is mature and these are assumed to be parts of the same gathering and are presumed isotypes.

Some commentary on the names listed among the synonyms may aid further investigators. The holotype material of *Discina pululahuana* is housed at FH but is in poor condition; therefore it is recommended that the slide that accompanies this specimen be examined. We confirmed that tissues and striate ascospores are identical to those of *C. venezuelae* as was suggested by Pfister (1974). Two specimen numbers are mentioned in the original description of *D. epixyla*, 249 and 527 and both specimens are in FH. **We designate here specimen number 249 as the lectotype of *D. epixyla*,** since number 527 is immature. The designated lectotype has a drawing by Patouillard of three ascospores on the packet. Patoulliard (in Duss 1903) noted that this species was very close to *D. pululahuana*, mentioned above, the differences being that the latter had larger ascomata and ascospores, as well as a reddish color. This was confirmed in examination of material, our measurements of ascospores of *D. pululahuana* are 40 x 14 (though they are described as 35–42 x 12–16 µm) and *D. epixyla* is reported as being 33 x 13–15 µm. We consider these differences irrelevant. Dennis (1954) first suggested that *D. epixyla* might be a synonym. Pfister (1974) examined Patouillard's specimen and proved that Dennis was correct.

General Notes: Seaver (1913,1928) treated this species as *Cookeina colensoi.* Le Gal (1953) pointing out that the ascospores of *C. colensoi* are smooth whereas those in *C. venezuelae* are distinctively marked with longitudinal ribs as Seaver has described for West Indian collections. Korf (1971, 1973) placed *C. colensoi* in the genus *Boedijnopeziza*, a previously monotypic genus. Based on a study of the specimens Korf had at hand, Pfister (1974) determined that Korf, like Seaver had confused *C. colensoi* and *C. venezuelae*. This is understandable since both species are hairless and have stipes. Both also have gelatinous material in the inner ectal excipulum. The recognition of the genus *Boedijnopeziza* has not been accepted by Le Gal (1953), Denison (1967), Pfister (1974), Pfister & Kaushal (1984) and Weinstein et al. (2002). Le Gal states that the shape and size of ascospores in *C. venezuelae* and *C. colensoi* are similar, but there are differences between them with regard to the ornamentation and appiculations. The spores of *C. colensoi* are smooth and have apiculate ascospores; *C. venezuelae* has striate non-apiculate ascospores. Le Gal illustrates *C. venezuelae* ascospores as narrowing at the poles, but with no apiculum present, whereas her illustrations of *C. sulcipes*, and *C. colensoi* show apiculi formed by a [stated by her as a mucilaginous] perispore. In some cases the perispore layer is uniform as Le Gal states, but we disagree with her, since we have observed that in other cases the perispore layer thickens clearly at the poles forming apiculi (one at each end) which seem to be formed by wall material and not gel.

Specimens examined. COLOMBIA: Trail between Hacienda Cincinnati and Jamonical, 100-1250m, 12 Aug 1935, *G. W. Martin 3312* (NY). ECUADOR. Sur la terre [probably on buried wood or wood fragments], Cratère de Pululahua, Lagerheim, Equador, Feb 1892 (holotype of *Discina pululahuana*) (FH-Pat). GUADELOUPE: Sur loutes sartes de branches mortes, Bois de la Riviére Saint-Louis, Guadeloupe, Fèvrier 1901, Duss 249, [Lectotype of *Discina epixyla*] (FH-Pat); Camp Jacob, sur bois pourri jeune coffee arabica, Fèv 1902, *527* [paratype of *Discina epixyla*] (FH-Pat) [as *Peziza* peut-être *Discina epixyla*]; Haut Matouba, Victor Hughes Trail, 700-1071m, on twigs and fallen branches, 9 Jan 1974, *D. H. Pfister, S. Carpenter, M. Sherwood 1161* (FH); Grand Matouba, Victor Hughes Trail, 700m, on twigs, 8 Jan 1974, *D. H. Pfister, S. Carpenter, M. Sherwood 1107* (FH); Saint Claude, Camp Jacob, 500-550m, on twigs and larger pieces of wood, 7 Jan 1974, *D. H. Pfister, S. Carpenter, M. Sherwood 1033* (FH); Montagne de St. Louis, 1902, *P. Duss* (NY). JAMAICA. Chester Vale, 3000-4000ft, 21-24 Dec 1908, *W. A. Murrill & Edna L. Murrill 349* (NY); Chestervale, 3000ft, 7 Feb 1903, *L. M. Underwood 1160* (NY); Morce's Gap, 5000ft, 29-30 Dec 1908, 2 Jan 1909, *W. A. & Edna L. Murrill 669* (NY), infertile but with asci; Rose Hill, 3500ft, on dead wood, 20 Oct-24 Nov 1902, *F. S. Earle 51* (NY); On wood, along trail between Woodcutter's Gap and ruins of Major Wallin's House, vicinity of Newcastle, Portland, Parish, 9.I.1971, *R.P. Korf et al.* (CUP-MJ-139); On twigs, along Lady's Mile Trail to just south of Woodcutter's Gap, vicinity of Newcastle, border of St. Andrew and Portland Parishes, 9.I.1971, *R.P. Korf et al.* (CUP-MJ-146, CUP-MJ-176); along Ulster Road Trail, Newcastle, St. Andrew Parish, 9.I.1971, *R.P.Korf et al.* (CUP-MJ-197); On wood, *Cecropia peltata* and other substrates, Chesterville Youth Development Camp, above Newcastle, St. Andrew Parish, 8.I.1971, *R.P.Korf et al.* (CUP-MJ-1, OSC 30140); On wood, near Dick's Pond, west of Hardwar Gap, near Holywell Recreation Area, St. Andrew Parish, elev. 2800-3000', 11.I.1971, *R.P.Korf et al.* (CUP-MJ-326); On twigs, Cinchona Botanical Gardens, St. Andrew Parish, elev. 4750', 8.I.1971, *R.P.Korf et al.* (CUP-MJ-53); On twigs, Cinchona Botanical Gardens, St. Andrew Parish, elev. 4750', 8.I.1971, *R.P.Korf et al. (CUP-MJ-47).* PANAMA. CHIRIQUI: Llanos del Volcan, 1100-1200m, in forest, 13 Jul 1935, *G. W. Martin 2782* (FH). PUERTO RICO: Cordillera Central, Toro Negro, Mun. de Juan Diaz, Long. 66°32'8" Lat. 18°9'10", on big log, 24 Jun 1996, *S. A. Cantrell 3381* (FH). VENEZUELA. On the ground, unlocalized, year 1855, Leg. *A. Fendler 282*, Venezuela (holotype of *Peziza venezuelae*) (K); Venezuela, *Fendler 282*, (isotype of *Peziza venezuelae*) Curtis Herbarium (FH); *Fendler* [probably an isotype of *Peziza venezuelae,* but with no number,], in an immature specimen with asci but no ascospores (Sprague Herbarium-FH), ; *Fendler* (NY) [as *Peziza venezuelae, Cookeina colensoi*], [probably an isotype of *Peziza venezuelae,* but with no number,], Ellis (NY); AMAZONAS: Sobre ramita caida, 25 Oct 1997, *T. Iturriaga 6065* (FH); ARAGUA: Maracay, Camino de Interpretación de la Naturaleza 'Andy Fields', Parque Nacional Henry Pittier, Estación Biológica Rancho Grande, sobre corteza de madera, 24 Nov 1994, *T. Iturriaga, L. Bracamonte, L. Ryvarden, O. Holmquist 2257* (VEN); Maracay, Camino de Interpretación de la Naturaleza 'Andy Fields', Estación Biológica Rancho Grande, Parque Nacional Henry Pittier. DISTRITO FEDERAL: On unidentified mossy log, trail from Quebrada Mariperez, through Vivero El Cuno and El Papelon to ca. 1 km. below Hotel Humboldt, El Avila, Parq. Nac. El Avila, Dto. Fed., *K.P. Dumont (VE-6194), R.F. Cain & G.J. Samuels,* 27.VII.1972 (NY); On unidentified wood, along trail 1-2 km above las [Los] Venados, El Avila, Parq. Nac. El Avila, Dto. Fed., *K.P. Dumont (VE-5828), R.F. Cain, G.J. Samuels & B. Manara,* 24.VII.1972 (NY); On unidentified wood, vicinity refugio "No te Apures", south facing slope of La Silla, Parq. Nac. El Avila, Edo. Miranda [Dto. Fed.], *K.P. Dumont (VE-3810), G.J. Samuels & B. Manara,* 30.VI.1972 (NY); On unidentified wood, between refugio "No te Apures" and Quebrada Los Palos Grandes, south facing slope of La Silla, Parq. Nac. El Avila, Edo. Miranda [Dto. Fed.], *K.P. Dumont (VE-3743), G.J. Samuels & B. Manara,* 30.VI.1972 (NY). MIRANDA: Baruta, Sartenejas, sobre corteza rama caida, Oct 1997, *T. Iturriaga 6066* (immature), *6034* (FH); Baruta, Sartenejas, sobre corteza rama caida, Oct 1997, O. del Guidice *6033* (FH).

Misapplied Names, Synonyms, and Doubful or Excluded Species

General note: There are many *Trichoscypha* species that are not accounted for in this synonymy list. Most are combined in the genus *Trichoscyphella*, a genus of the *Hyaloscyphaceae*.

abnormis – *Pilocratera abnormis* Peck, N. Y. St. Educ. Dept. Bull 495: 37. 1911.

This is on *Betula* in New York and is an inoperculate discomycete.

afzelii – *Peziza afzelii* Fr. = *Cookeina speciosa*

aluticolor – *Peziza aluticolor* Berk. = *Cookiena colensoi*

amoena – *Peziza amoena* Lév. = *Cookeina speciosa*

antillarum – *Peziza venezuelae* var. *antillarum* Pat. in Duss, Énum. Champ. Guadeloupe, p. 64. 1903. [sur le sol dans une cafèyere FH) !

This is *Phillipsia domingensis* (Berk.) Berk.

beccariana – *Peiza hindsii* var. *beccariana* Ces. = *Cookeina speciosa*

argentinensis – *Ciboria argentinensis* Speg. = *Cookeina colensoi*

calyciformis – *Trichoscypha calyciformis* (Willd.) Grélet, Rev. Mycol. (Paris) (NS) 16: 87. 1951.

This is an inoperculate discomycete.

calycina – *Trichoscypha calycina* (Schumach.) Vuill., Bull. Soc. Mycol. Fr. 1: 117. 1885.

This is an inoperculate discomycete.

celebica – *Pilocratera tricholoma* var. *celebica* Henn. = *Cookeina tricholoma*

ciborioides – *Geopyxis ciborioides* Starbäck = *Cookeina colensoi*

colensoi – *Cookeina colensoi* (Berk.) Seaver sensu Seaver = *Cookeina venezuelae*

crocina – *Peziza hindsii* var. *crocina* (Mont. & Fr.) Cesati, Atti Reale Accad. Sci. Fis. 8: 11. 1878 [1879].

This small species, originally described from France as *Peziza crocina* is mostly likely referable to a group of inoperculate discomycetes.

discifera – *Cookeina discifera* (Haszl.) Kuntze, Rev. gen. Pl. 849.1891 ≡ *Craterium disciferum* Hazsl., Verh. K.K. zool.- Bot. Ges. Wien 37: 167. 1887) ≡ *Trichoscypha discifera* (Hazsl.) Sacc., Syll. fung. 8:163. 1889 ≡ *Pilocratera discifera* (Hazsl.) Sacc. & Traverso, Syll. fung. 20: 412. 1911.

This is a species of *Helvella* judging by the illustration and since the material is from Hungary, an unlikely location for a species of *Cookeina* to occur, it is excluded.

elata – *Geoyxis elata* Massee = *Cookeina speciosa*

engleriana – *Pilcratera engleriana* Henn. = *Cookeina speciosa*

epixyla – *Discina epixyla* Pat. = *Cookeina venezuelae*

fusca – *Cookeina sulcipes* var. *fusca* Alas. = *Cookeina speciosa*

fusispora – *Sarcoscypha fusispora* Sawada, Special Publication, College of Agriculture, National Taiwan University 11: 49. 1959.

Published without a Latin description or diagnosis this is a nomen nudum that refers to *Cookeina insititia*.

globosa – *Cookeina globosa* Douanla-Meli = *Cookeina speciosa*

This taxon was described and illustrated with a fringe of marginal hairs as in *C. speciosa* but as having globose to subglobose ascospores. Our attempt to obtain the specimen has been unsuccessful. Judging by the illustrations and descriptions it seems clear that this species is based on immature specimens of *C. speciosa*. The spores are small 12-15 x

10-12 µm and thin-walled. Since asci mature simultaneously it is often possible, with a single collection as is the case here, to misjudge maturation. Without the specimen our recourse has been to treat this as a synonym of *C. speciosa*.

hindsii – *Peziza hindsii* Berk. = *Cookeina speciosa*

hystrix – *Peziza hystrix* Berk. = *Cookeina tricholoma*

insititia – *Peziza insititia* ≡ *Cookeina insititia*

javanica – *Peziza javanica* Nees ex Lév. = *Cookeina speciosa*

magnispora – *Trichoscypha magnispora* Lloyd = *Cookeina insititia*

maxima – *Pilocratera maxima* P. Syd. = *Cookeina speciosa*

minor – *Peziza tricholoma* var. *minor* Mont. = *Cookeina tricholoma*

medusina – *Peziza medusina* Speg. = *Cookeina tricholoma*

moelleriana – *Geopyxis moelleriana* Henn. = *Cookeina colensoi*

mundkurii – *Cookeina mundkurii* S. C. Kaushal, J. Indian Bot. Soc. 65: 405. 1986.

Specimens of this species have not been available to us for study. Based on the description it is likely that this represents a collection of *C. indica*. It agrees with that species in lacking obvious hairs, in the long ellipsoidal ascospores with longitudinal striations that do not anastomose. The name *C. indica* (Pfister & Kaushal 1984) has priority and *C. mundkurii* is considered a synonym.

notarisiana – *Peziza notarisiana* Bagnis, Atti Reale Accad. Lincei 8: 15. 1876– 1877. *Trichoscypha notarisiana* (Bagnis) Sacc., Syll. fung. 8: 162. 1889. ≡ *Cookeina notarisiana* (Bagnis) Kuntze, Revis. gen. pl. 849. 1891. ≡ *Pilocratera notarisiana* (Bagnis) Sacc. & Traverso, Syll. fung. 20: 413. 1910.

This is an inoperculate discomycete.

novoguianensis – *Pilocratera novo-guianensis* Ramsb. = *Cookeina speciosa*

pululahuana – *Discina pululahuana* Pat. = *Cookeina venezuelae*

sessilis – *Ciboria sessilis* Starbäck = *Cookeina colensoi*

sphaeroidospora – *Boedijnopeziza sphaeroidospora* Y. Otani = *Cookeina insititia*

striatospora – *Geopyxis striatospora* Maubl. & Roger

This is a nomen nudum; the description refers to *Cookeina speciosa*.

striispora – *Peziza striispora* Ellis & Everh. = *Cookeina tricholoma*

subfloccosa – *Plectania subfloccosa* Hazsl. Magyar. Discomyc., tab. 5 fig. 29 ≡ *Pilocratera subfloccosa* (Hazsl.) Sacc. & Traverso, Syll. fung. 20: 413. 1911.

sulcipes – *Peziza sulcipes* Berk. = *Cookeina speciosa*

sumatrana – *Cookeina sumatrana* Boedijn = *Cookeina speciosa*

tetraspora – *Cookeina tetraspora* Seaver, Mycologia 17: 45. 1925 ≡ *Phillipsia tetraspora* (Seaver) Le Gal, Prodr. Flore Mycol. Madagascar, 262. 1953. ≡ *Sarcoscypha tetraspora* (Seaver) Denison, Rev. Biol. Trop. 11: 107. 1963. ≡ *Nanoscypha tetraspora* (Seaver) Denison, Mycologia 64:619. 1972.

This species is the type species of *Nanoscypha* Denison (1972).

tricholoma – *Peziza tricholoma* Mont. ≡ *Cookeina tricholoma*

viridirubescens – *Trichoscypha viridirubescens* (Bagnis) Sacc., Syll. fung. 8: 162. 1889. ≡ *Peziza viridirubescens* Bagnis, Atti Reale Accad. Lincei 8: 15. 1876-1877. ≡ *Cookeina viridirubescens* (Bagnis) Kuntze, Revis. gen. pl. 2: 849. 1891. ≡ *Pilocratera viridirubescens* (Bagnis) Sacc. & Traverso, Syll. fung. 20: 413. 1911.

This is an inoperculate discomycete.

Acknowledgments

This work was supported by NSF grants DEB-9521944 and DEB-0315940 to Donald Pfister and by a grant from the David Rockefeller Center for Latin American Studies of Harvard University which supported Teresa Iturriaga during an extended study leave at Harvard. We wish to thank Richard N. Weinstein, for work done on the project during a post-doctoral fellowship, and Karen Hansen, with whom we were able to discuss various aspects of the project. Richard Korf and Amy Rossman reviewed a draft and Jack D. Rogers and Sharon Cantrell served as formal reviewers. Shaun Pennycook provided us with valuable nomenclatural and technical advice. We thank Jens Petersen, Thomas Læssøe, and Roy Halling for photographs. We owe a debt to the curators of the following herbaria who allowed access to specimens: B, BPI, CUP, K, NY, PC, OSC, PDD, S, USB, VEN. The authors acknowledge the inspiration of Richard P. Korf, their professor at Cornell, to whom they dedicate this paper.

Literature cited

Alexopoulos CJ, Butler EE. 1949. Conidia-like structures in *Plectania coccinea*. Mycologia 41: 180-182.

Alasoadudra SO. 1972. Studies in the higher fungi of Nigeria. IV. Some operculate discomycetes. Nova Hedwigia 23: 767-780.

Arpin N. 1969. Les caroténoïdes d'Discomycètes: Essai chimiotaxinomique. Bull. Mens. Soc. Linn. Lyon, Suppl. 38: 1-169

Bentham G, Hooker JD. 1862. Genera plantarum ad exemplaria imprimis in herbariis kewensibus servata definita. Vol. 1(1). A. Black. London. Pp 1-434.

Berkeley MJ. 1852. Enumeration of some fungi from St. Domingo. Ann. Mag. Nat. Hist. series II. 9: 192-203.

Berkeley MJ, Broome CE. 1875. Enumeration of the fungi of Ceylon. J. Linn. Soc. 14: 29-140.

Berthet P. 1964. Essai Biotaxinomique sur les Discomycètes. Joanny Lorge. Lyon.

Bi Z-s, Zheng G-y, Li T-h. 1993. The Macrofungus Flora of China's Guangdong Province 734 p. Hong Kong, Chinese University Press.

Boedijn KB. 1929. Beitrag zur Kenntnis der Pilzflora von Sumatra. Recueil. Trav. Bot. Néerl. 26: 396-439.

Boedijn KB. 1933. The genera *Phillipsia* and *Cookeina* in Netherlands India. Bull. Jard. Bot. Buitenzorg. 13: 57-76

Boedijn KB. 1951. Some mycological notes. Sydowia 5: 211-229.

Boudier JLE. 1885. Nouvelle classification naturelle des discomycètes charnus connus généralement sous le nom de pezizes. Bull Soc Mycol France 1: 91-120.

Cabello MN. 1988. Estudio sistematico del suborden *Sarcoscyphineae* (*Pezizales,* Ascomycotina) empleando tecnicas numericas. Bol. Soc. Argent. Bot. 25: 395-413.

Calonge FD. 1986. Ilustraciones de hongos y liquenes de la expedicion botanica a Nueva Granada, depositadas en le real Jardin Botanico de Madrid. Caldasia 15: 47-51.

Chacón, S. 1988. Conocimiento etnoecologico de los hongos en Plan de Palmar, Municipio de Papantla, Veracruz, Mexico. Mic. Neotrop. Aplic. 1: 45-54.

Chacón, S, Medel R. 1990. Ascomycetes little known in Mexico V. Description of some *Pezizales.* Revista Mex. Micol. 6: 199-206.

Cooke MC. 1874. Carpology of *Peziza*. Grevillea 3: 30-31, pl. 27-30.

Cooke MC. 1875. Carpology of *Peziza*. Grevillea 3:186, pl. 43-44.

Cooke MC. 1879. Mycographia, seu Icones fungorum. I. Discomycetes. Part I. Williams & Norgate, London.

Cooke MC. 1892. Handbook of Australian Fungi. William & Norgate. London. 456 pp.

Denison WC. 1963. A preliminary study of the operculate cup-fungi of Costa Rica. Rev. Biol. Trop. 11: 99-129.

Denison WC. 1967. Central American *Pezizales*. II. The genus *Cookeina*. Mycologia 59: 306-317.

Denison WC. 1972. Central American *Pezizales*. IV. The genera *Sarcoscypha*, *Pithya*, and *Nanoscypha*. Mycologia 64: 609-623.

Dennis RWG. 1954. Operculate Discomycetes from Trinidad and Jamaica. Kew Bull. 9: 417-421.

Dennis RWG. 1970. Fungus flora of Venezuela and adjacent countries. Kew Bull. Additional Ser. 3. 531 pp.

Dennis RWG. 1994. Plumier's Discomycetes. Mycotaxon 51: 237-239.

Dijk H van, Onguene NA, Kuyper TW. 2003. Knowledge and utilization of edible mushrooms by local populations of the rain forest of south Cameroon. Ambio 32: 19-23.

Douanla-Meli, C. Langer E. 2005. Notes on discomycetes (*Helotiales, Pezizales)*: New species and new records from Cameroon. Mycotaxon 92: 223-237.

Durrieu G, He Q, Chaumeton J-P, Chauveau C. 1997. *Cookeina insititia*, a new Discomycetes from China. Acta Bot. Yunnanica. 19: 128-120.

Duss A. 1903. Énumération méthodique des champignons recueillis a la Guadeloupe et a la Martinique. Fungi by N. T. Patouillard. Lons-le-Saunier

Eckblad F-E. 1968. The genera of operculate discomycetes. A re-evaluation of their taxonomy, phylogeny and nomenclature. Nytt Mag. Bot. 15: 1-191.

Ferdinandsen C, Winge Ø. 1910. Fungi from prof. Warmings expedition to Venezuela and the West-Indies. Dansk Tidsskr. 30: 208-222.

Fries EM. 1822. Systema Mycologicum sistens fungorum ordines, genera et species huc usque cognitas. 2 part 1. Griefswald.

Fries EM. 1860. Reliquiae Afzelianae, sistentes icons fungorum quos in Guinea collegit et in aere incises excudi curavit Adamus Afzelius. Upsaliae. Edquist & Soc.

Gamundí IJ. 1957. El genero *Cookeina* en la República Argentina. Bol. Soc. Argent. Bot. 6: 212-222.

Gamundí IJ. 1959. Addenda a las especies Argeninas de *Cookeina* Kuntze. Bol. Soc. Argent. Bot. 7: 201-204.

Gamundí IJ. 1971 Algunos discomycetes de Chile. Bol. Soc. Argent. Bot. 13: 260-289.

Gamundí IJ. 1983. *Pezizales* de America Tropical. Pp. 151-171 in Anais [Congresso Nacional de Botânica] 34 congresso, v.1.

Hanlin RT. 1993. *Cookeina sulcipes* - an addendum. Mycotaxon. 46: 403-405.

Hanlin RT, Chien C-Y, Tortolero O. 1991. Icones Ascomycetum Venezuelae: *Cookeina sulcipes*. Trans. Mycol. Soc. R. O. C. 6: 45-53

Harrington FA. 1990. *Sarcoscypha* in North America (*Pezizales, Sarcoscyphaceae*). Mycotaxon. 38: 417-458.

Harrington FA, Pfister DH, Potter D, Donoghue MJ. 1999. Phylogenetic studies within the Pezizales. I. 18S rDNA sequence data and classification. Mycologia 91: 41-50.

Hennings P. 1892 [1891]. Fungi africani. Bot. Jaarb. Syst. 14: 337-373.

Hood IA. 1992. An Illustrated Guide to Fungi on Wood in New Zealand. Auckland University Press. Aukland.

Hooker JD 1855. The botany of the Antarctic voyage…II. Flora novae-zelandiae. London. Authoriy of the Lord Commissioners of the Admiralty.

Ito S, Imai S. 1937. Fungi of the Bonin Islands II. Trans. Sapporo Nat. Hist. Soc. 15: 52-59.

Kaushal SC. 1986. A new species of *Cookeina* (*Pezizales*) from India. J. Indian Bot. Soc. 65: 404-407.

Korf RP. 1951. A monograph of the *Arachnopezizeae*. Lloydia 14: 129-180.

Korf RP. 1970. Nomenclatural notes. VII. Family and tribe names in the *Sarcoscyphineae* (Discomycetes) and a new taxonomic disposition of the genera. Taxon 19: 782 - 786.

Korf RP. 1971. Some new Discomycete names. Phytologia 21: 201-207.

Korf RP. 1972. A synoptic key to the genera of the *Pezizales*. Mycologia 64: 937-994.

Korf RP. 1973. Discomycetes and *Tuberales*. In: Ainsworth GC, Sparrow FK, Sussman AS, eds. The fungi: an advanced treatise. Vol 4A. New York: Academic Press. p 249 - 319.

Kuntze O. 1891. Revisio Generum Plantarum 2: 375-1011.

Le Gal M. 1953. Le Discomycètes de Madagascar. Prodr Flore Mycol Madagascar 4: 1-465.

Le Gal M. 1960. Flore iconographique des champignons du Congo dédiée a sa majesté Lépold III. Illustrée en couleurs par Mme. M. Goossens-Fontana. 9: 167-183.

Le Gal M. 1969. Position taxinomique du genre *Phaedropezia* Le Gal et révision de la famille des *Humariaceae*. Bull. Soc. Mycol. France. 85: 5-19.

Lloyd CG. 1921. *Trichoscypha magnispora* from Otto A. Reinking, Philippines.Mycological Writings 6: 1050.

Maldonado González S. 2000. El género *Cookeina* Kuntze (Ascomycota) en Cuba. Revista, Jard. Bot. Nac. Univ Habana. 21: 305-307.

Massee G. 1896. Redescription of Berkeley's types of fungi. J. Linn. Soc. Bot. 31: 462-525.

Massee G. 1898. Fungi exotici, I. Kew Bull. 1898: 113-136.

Melendez-Howell LM. 1986. Les champignons dans la degradation de la forêt primaire de L'Arataye. Mem. Mus. Nat. Hist. Nat. (Paris), A 132: 257-261.

Melendez-Howell LM, Mascarell G, Bellemere A. 2003. Etudes ultrastructurales dans le genre *Cookeina* (Ascomycetes, *Peizales, Sarcoscyphaceae*). Mycotaxon 87: 53-80.

Montagne C. 1834. Nouvelles espèces de cryptogames. Ann. Sci. Nat. Bot. ser. 2 2:77.

Moravec, J. 1997. Discomycetes of Madagascar – I. *Phillipsia ranomafanensis* sp. nov. and ascospore sculpture of *Cookeina colensoi* proved by SEM (Discomycetes, *Pezizales, Sarcoscyphaceae*). Czech Mycol. 50: 21 – 33.

Nagao H. 1997. Materials for the fungus flora of Japan (51). Mycoscience 38: 351-353.

Otani Y. 1971. Mycological reports from New Guinea and the Solomon Islands. 3. Enumeration of the *Sarcoscyphaceae* and *Scutellinia* (*Humariaceae*). Bull. Nat. Sci. Mus., Tokyo 14: 401-422.

Otani Y. 1975. Discomycetes collected in Papua New Guinea pp. 5-41 in Reports on the cryptogams in Papua New Guinea, The party 1973-'74, of The botanical expedition to Papua New Guinea. The National Science Museum, Tokyo.

Paden JW. 1975. Ascospore germination, growth in culture, and imperfect spore formation in *Cookeina sulcipes* and *Phillipsia crispata*. Canad. J. Bot. 53: 56-61.

Paden JW. 1984. A new genus of Hyphomycetes with teleomorphs in the *Sarcoscyphaceae* (*Pezizales, Sarcoscyphineae*). Canad. J. Bot. 62: 211-218.

Pfister DH. 1973. Notes on Caribbean Discomycetes. III. Ascospore germination and growth in culture of *Nanoscypha tetraspora* (*Pezizales, Sarcoscyphineae*). Mycologia 65: 952-956.

Pfister DH. 1974. Notes on Caribbean Discomycetes. IV. *Cookeina venezuelae*, *C. colensoi* and the genus *Boedijnopeziza*. Phytologia 27: 55-62.

Pfister DH. 1978a. Apothecial development in *Cookeina tricholoma* with comments on some related species. Mycologia 70: 1253-1257.

Pfister DH. 1978b. Cryptogams of the United States North Pacific Exploring Expedition, 1853-1856. Farlow Reference Library and Herbarium. Cambridge, Ma

Pfister DH, Kaushal R. 1984. *Cookeina indica*, a new species from India with a key to the species of *Cookeina*. Mycotaxon 20: 117-121.

Pfister DH, Kimbrough JW. 2001. Discomycetes pp. 257-281 in The Mycota, eds. McLaughlin, McLaughlin & Lemke. Springer-Verlag, Berlin, Heidelberg.

Pfister DH, Kurogi S. 2004. A note on some morphological features of *Chorioactis geaster* (*Pezizales*, Ascomycota). Mycotaxon 89: 277-281.

Plumier C. 1705. Traité des fougères de l'Amerique. Paris. 146 pp, 170 pl.

Rick J. 1931. Monographia Pezizinearum Riograndensium. Broteria Ser. Bot. 25: 77 - 98. 1931.

Rifai MA. 1968. The Australasian *Pezizales* in the herbarium of the Royal Botanic Gardens Kew. Verh. Kon. Ned. Akad. Wetensch., Afd. Naturk. Tweede Sect. 57: 1-295.

Romero AI, Gamundí IJ. 1986. Algunos Discomycetes xilofilos del area subtropical de la Argentina. Darwiniana 27: 43-63

Rosinski MA. 1953. Two types of spore germination in *Sarcoscypha coccinea* (Scop. ex Fr.) Lambotte. Mycologia 45: 302-306.

Saccardo PA. 1889. Sylloge fungorum. Discomycetum et Phymatosphaeriacearum. Vol. 8. 1143 p.

Samuelson DA 1975. The apical apparatus of the suboperculate ascus. Canadian Journal of Botany 53: 2660-2679.

Sánchez AD, Chacón S, Sánchez JE. 1993. Producción natural de *Cookeina sulcipes* (Ascomycotina, *Pezizales*) en la región de Tapachula, Chiapas (México). Rev. Mex. Mic. 9: 47-56.

Sánchez Vázquez JE, Huerta Palacios G, Calvo Bado LA. 1995. Progress in the cultivation of the edible mushroom *Cookeina sulcipes*. Pp. 857 – 862 in Science and Cultivation of Edible Fungi, vol. 2, T. J. Elliott ed. Balkema, Rotterdam.

Spegazzini C. 1902. Mycetes Argentinensis. Anal. Mus. Nac. Buenos Aires, ser. 3 1902: 49 – 89.

Seaver FJ. 1913. Some tropical cup-fungi. Mycologia 5: 185-193.

Seaver FJ. 1925. Studies in Tropical Ascomycetes – III. Porto Rican cup-fungi. Mycologia 17: 45-50.

Seaver FJ. 1927. A tentative scheme for the treatment of the genera of *Pezizaceae*. Mycologia 19: 86-89.

Seaver FJ. 1928. The North American cup-fungi (Operculates). Published by the author. New York

Seaver FJ. 1936. The genera *Phillipsia* and *Cookeina*. Mycologia 28: 90-92.

Seaver FJ. 1942. The North American cup-fungi (Operculates). Supplemented. Published by the author. New York.

Starbäck K. 1904. Ascomyceten der ersten Regnellschen Expedition, III. Ark. Bot. 2: 1-22.

Teng SC. 1963. Fungi of China. Beijing pp. 277-279.

Tobon LE. 1991. Ascomicetos de Colombia: Discomicetos del departamento de Antioquia. Caldasia 16: 327-336.

Villarreal L, Perez-Moreno J. 1989. Los hongos comestibles silvestres de Mexico, un enfoque integral. Micol. Neotrop. Apl. 2: 77-114.

Vooren N van. 2003. Deux discomycètes intéressants récoltés à La Martinique, note complémentaire. Bull. Mens. Soc. Linn. Lyon 72: 156-157.

Vooren N van, Lopez F. 2002. Deux discomycètes intéressants récoltés à La Martinique. Bull. Mens. Soc. Linn. Lyon 71: 152-156.

Wang Z. 1997. Taxonomy of *Cookeina* in China. Mycotaxon 62: 289-298.

Wang YZ. 2001. Discomycetes of the *Sarcoscyphaceae* in Taiwan. Mycotaxon 79: 329-336.

Weinstein RN, Pfister DH, Iturriaga T. 2002. A phylogenetic study of the genus *Cookeina*. Mycologia 94: 673-682.

Yang Z-l. 1990. Several noteworthy higher fungi from southern Yunnan, China. Mycotaxon 38: 407-416.

Zoberi MH. 1973. Influence of water on spore release in *Cookeina sulcipes*. Mycologia 65: 155-160.

MYCOTAXON

Volume 95, pp. 181–184 January–March 2006

A new nematode-trapping hyphomycete of *Arthrobotrys*

WEIFENG HU, YAN LI, MINGHE MO & KEQIN ZHANG*

*kqzhang111@yahoo.com.cn
Laboratory for Conservation and Utilization of Bioresources
Yunnan University, Kunming 650091, P.R. China

Abstract—*Arthrobotrys multisecundaria*, isolated from Yunnan, China, was illustrated and described as a new nematode-trapping fungus, which captures nematodes by means of adhesive three-dimensional networks. It is characterized mostly by one-septate primary, elliptical conidia, which produce unicellular secondary conidia from both distal and basal ends. The new species differs obviously from other related species by producing up to four secondary catenulate conidia.

Keywords—*Dactylella, Monacrosporium,* predacious fungi, taxonomy

Introduction

Nematophagous fungi have been the subject of research for several decades in the fundamental studies of microbiological ecology, distribution and systematics, and potential biological control agents of nematode pests of plants and animals (Liu & Zhang 2003). These fungi usually can be categorized into four types: endoparasitic, nematode-trapping, egg- and female-parasitizing, and toxin-producing fungi (Barron & Thorn 1987). Among the four types, nematode-trapping fungi (NTF) can produce various trapping-devices to capture nematodes and other small animals (Duddington 1951, Scholler et al. 1999, Ahrén et al. 2004). Traditionally, these predacious hyphomycetes were assigned to three genera (*Arthrobotrys, Dactylella, Monacrosporium*) according to the morphology of conidia (Cooke & Dickinson 1965). Trapping structures were used to rationalize the classification of the nematode-trapping fungi with the molecular data (Liou & Tzean 1997, Pfister 1997, Ahrén et al. 1998). Based on the analyses of partial 18S rDNA, ITS and 5.8S rDNA, Scholler et al. (1999) classified NTF into four genera: *Arthrobotrys, Dactylellina, Drechslerella* and *Gamsylella.* Li et al. (2005) redefined the systematic classification of nematode-trapping fungi based on phylogenies inferred from molecular analyses of 28S rDNA, 5.8S rDNA and β-tubulin genes, which indicated NTF should be divided into three genera: *Arthrobotrys, Dactylellina* and *Drechslerella.*

Materials and methods

Soil samples from Tengchong, Yunnan Province were sprinkled on corn meal agar (CMA) plates inoculated with free-living nematodes, *Panagrellus redivivus.* After approximately one month, a fungus forming adhesive three-dimensional networks was isolated, and identified as a new taxon after a detailed morphological study.

Based on the classification of NTF by Li et al. (2005), we assigned this new taxon to *Arthrobotrys* and here propose the name *Arthrobotrys multisecundaria*. Morphological distinctions between the new species and similar species are discussed.

Taxonomic Description

Arthrobotrys multisecundaria W.F. Hu & K.Q. Zhang **sp. nov.** **(Figs. 1-14)**

> *Coloniae in agaro CMA albidae, post 5 dies 25°C 2.5 cm diam. Mycelium sparsum, hyphis septatis, ramosis, 4–7.5 μm latae. Conidiophora erecta, hyalina, simplicia, 200–365 μm longa, 2.5 μm lata ad apicem. Conidia hyalina, ellipsoideas, 32.5-55×15-22.5μm, 1 septata vel non-septata. Conidia secundaria non-septata. Reticula tenacia quae vermiculos nematodeos capiunt evolventibus.*
>
> *Etymology:* The species name refers to the formation of conidia in a catenulate array.
>
> Holotype: YMF1.01821A, Tengchong, Yunnan Province, China, 2005, Weifeng Hu. The holotype and its culture (YMF1.01821) are deposited in the Laboratory for Conservation and Utilization of Bioresources, Yunnan University.

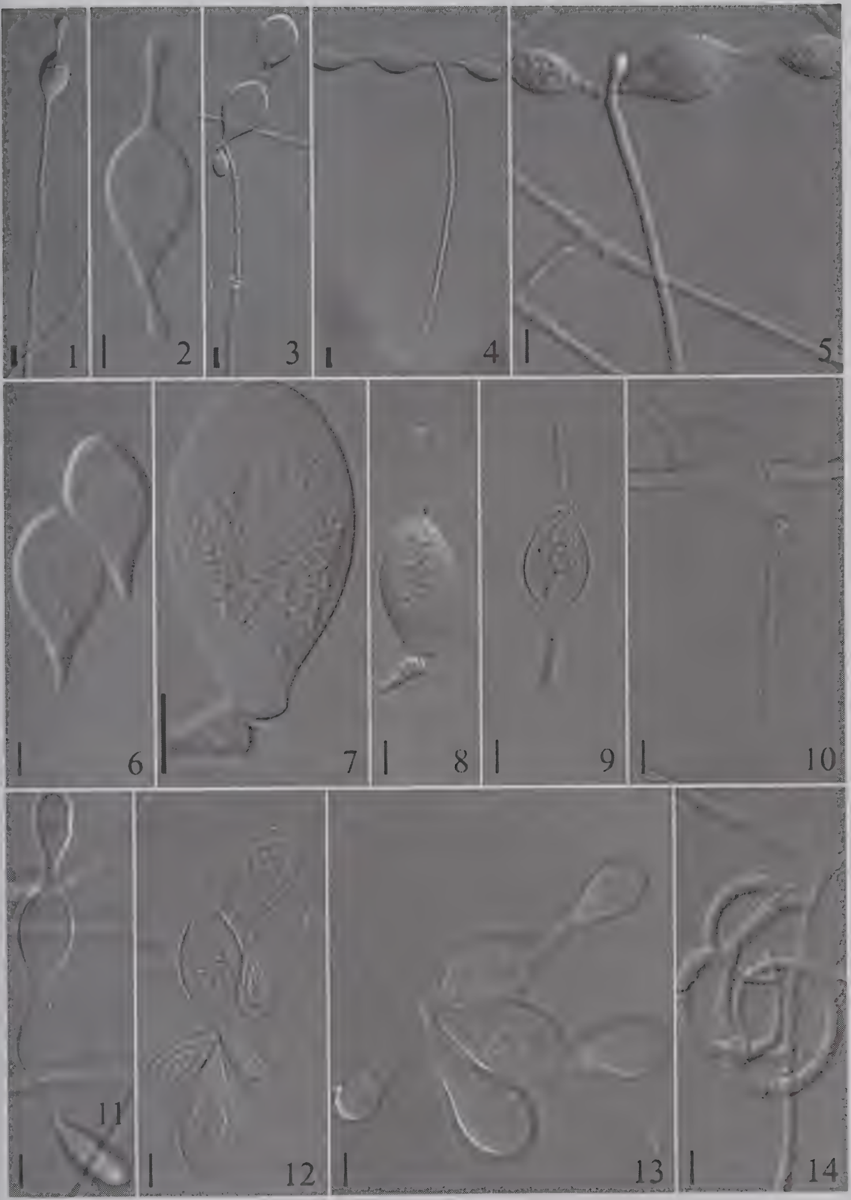
Colonies on CMA whitish, slowly-growing and extending a diameter of 2.5 cm at 25° within 5 days. Mycelium hyaline, scanty, vegetative hyphae, septate, branched, 4-7.5 μm wide. Conidiophores (Fig. 1, 3, 4-5) erect, hyaline, septate, unbranched, 200-365 μm long, 5 μm wide at the base, gradually tapering upward to a width of 2.5 μm at the apex, bearing one or two conidia. Conidia (Figs. 6-7) hyaline, ellipsoidal, 32.5-55 × 15-22.5 μm, 1-septate (75%) or non-septate (25%). Secondary conidia (Figs. 3-5, 11-13) can be produced from both distal and basal ends of primary conidia, non-septate, 32.5-55 × 15-22.5 μm. The predacious organ exhibits adhesive three-dimensional networks.

The single spore culture produced conidia after six days incubation on PDA at 25 °C. Each conidiophore produced one to two conidia (Figs. 4, 5, 9, 10, 13) that proliferated to form secondary conidia from both the distal and basal ends of primary conidia (Figs. 1, 2, 8).

This process sometimes was repeated two to three times so that three to four conidia could be produced on each conidiophore in catenulate arrangement (Fig. 3, 4, 11). Sometimes up to three conidia were produced from the distal end of a conidium (Fig. 12).

An interesting process was observed during our study: primary conidia were observed to dehisce at the septa to form germ tubes from the end of the distal cell (Figs. 7-8) while the basal cell remain attached to the distal cell (Fig. 10).

Comments-This species resembles *Monacrosporium indicum* (Chowdhry & Bahl) Xing Z. Liu & K.Q. Zhang (1994) and *M. janus* S.D. Li & Xing Z. Liu (Li et al. 2003) in conidial shape and number of septa but differs in conidial size and method of conidial germination. Additionally, in *M. indicum*, conidia are elliptic, obovoid or top-shaped, 22-30×14-20μm, and mostly 2-septate with distinct hila. In *M. janus*, conidia are broadly turbinate to napiform, 15-26 × 17.5-37.5 μm, 1-2-septate (mostly 1 septa). *A. multisecundaria* is distinguished by the catenulate conidial arrangement and germination that occurs from both basal and distal ends of conidia, characters that were not observed in *M. indicum, M. janus,* or other related nematode-trapping fungi.


1
2
3
4
5
6
7
8
9
10
11
12
13
14

Figs. 1-14. *Arthrobotrys multisecundaria.* Figs. 1, 3-5, 10. Conidia on conidiophores. Figs. 2, 6-9, 11-13. Primary conidia with secondary conidia. Fig. 14. Adhesive three-dimensional networks. Bars = 10 µm

Acknowledgments

We wish to thank Dr Xingzhong Liu and Dr Shidong Li for their suggested revisions of the manuscript. This work was supported by the project from the Department of Science and Technology of Yunnan Province, P. R. China (2005NG05).

Literature Cited

Ahrén D, Faedo M, Rajashekar B, Tunlid A. 2004. Low genetic diversity among isolates of the nematode-trapping fungus *Duddingtonia flagrans*: evidence for recent worldwide dispersion from a single common ancestor. Mycol. Res. 108: 1205-1214.

Ahrén D, Ursing BM, Tunlid A. 1998. Phylogeny of nematode trapping fungi based on 18r DNA sequences. FEMS Microbiology Letters 158: 179-184.

Barron GL, Thorn RG. 1987. Destruction of nematodes by species of *Pleurotus*. Can. J. Bot. 65: 774-778.

Cooke RC, Dickinson CH. 1965. Nematode-trapping species of *Dactylella* and *Monacrosporium*. Trans. Br. Mycol. Soc. 48: 621-629.

Duddington CL. 1951. The ecology of predacious fungi. I. Preliminary survey. Trans. Br. Mycol. Soc. 34: 322-331.

Li SD, Miao ZQ, Zhang YH, Liu XZ. 2003. *Monacrosporium janus* sp. nov. a new nematode-trapping hyphomycete parasitizing sclerotia and hyphae of *Sclerotinia sclerotiorum*. Mycol. Res. 107: 888-894.

Li Y, Hyde KD, Jeewon R, Cai L, Vijaykrishna D, Zhang KQ. 2005. Phylogenetics and evolution of nematode-trapping fungi estimated from nuclear & protein coding genes. Mycologia (in press)

Liou GY, Tzean SS. 1997. Phylogeny of the genus *Arthrobotrys* and allied nematode-trapping fungi based on rDNA sequences. Mycologia 89: 876-884.

Liu XF, Zhang KQ. 2003. *Dactylella shizishanna* sp. nov. from Shizi Mountain. China.Fungal Diversity 14: 103-107.

Liu XZ, Zhang KQ. 1994. Nematode-trapping species of *Monacrosporium* with special reference to two new species. Mycol. Res. 98: 862-868.

Pfister DH. 1997. Castor, Pollux and life histories of fungi. Mycologia 89: 1-23.

Scholler M, Hagedorn G, Rubner A. 1999. A reevaluation of predatory orbiliaceous fungi. II. A new generic concept. Sydowia 51: 89-113.

MYCOTAXON

Volume 95, pp. 185–188 January–March 2006

Hyphodontia tubuliformis, a new species from Taiwan

SHENG-HUA WU

shwu@mail.nmns.edu.tw
Department of Botany, National Museum of Natural Science
Taichung, Taiwan 40419, Republic of China

Abstract—*Hyphodontia tubuliformis*, collected from *Pinus* sp. in subtropical northern Taiwan, is described as new to science. This new species is characterized by having tubular cystidia and cylindrical basidiospores. A description and line drawing are provided for this new taxon.

Key words—*Aphyllophorales*, Basidiomycota, taxonomy, wood-decaying fungi

Introduction

The genus *Hyphodontia* J. Erikss. is a corticioid member of the Homobasidiomycetes, with about one hundred species (including *Alutaceodontia* Hjortstam & Ryvarden and *Kneiffiella* P. Karst.) known worldwide (Parmasto et al., 2004). *Hyphodontia* spp. are wood-decaying saprobes, causing a white rot in wood. Molecular evidence (Larsson et al., 2004; Binder et al., 2005) shows that *Hyphodontia* belongs to the hymenochaetoid clade of Homobasidiomycetes. Previous surveys of *Hyphodontia* spp. include those of Lin & Chen (1990), Wu (1990), Langer (1995), Wu (2000) and Wu (2001). This study adds a new species of *Hyphodontia* collected from subtropical northern Taiwan.

Materials and Methods

Free-hand thin sections of the basidiocarp were prepared for microscopic studies. For observations and measurements of microscopic characters, 5% KOH was used as a mounting medium to ensure rehydration. Melzer's reagent (IKI) was employed to detect amyloidity and dextrinoidity. Cotton blue (CB) was used as a mounting medium to determine cyanophily.

Taxonomy

Hyphodontia tubuliformis Sheng H. Wu, **sp. nov.** (Fig. 1)

Basidiocarpus effusus, submembranaceo-pelliculus, 80-200 µm crassus; superficies hymenialis grandinioideo-odontoidea. Systema hypharum monomiticum; hyphae fibulatae. Cystidia tubuliformia. Basidia suburniformia, 7-13 x 3.3-4 µm, 4 sterigmatibus. Basidiosporae cylindricae, laeves, tenuitunicatae, 4.7-5.5 x 1.8-2.2 µm, IKI-, CB-.

Etymology. From *tubuliformis* (= tubule-like), referring to the shape of cystidia in this species.

Basidiocarp resupinate, effuse, submembranaceous-pellicular, fairly soft, 70-200 µm thick in section (aculei excluded). Hymenial surface cream, grandinioid-odontioid, not cracking; margin thinning out, arachnoid, concolorous. Aculei separate or fused, > 10 per mm, conical or subulate, up to ca. 120 µm long and 60 µm wide.Hyphal system monomitic; hyphae nodose-septate. Subiculum with fairly loose texture; hyphae moderately ramified, colorless, ± vertically oriented, distinct and fairly straight, 2.5-4.5 µm diam., hyphal walls from slightly thickened to 1.2 µm thick. Hymenium somewhat thickening, with dense texture; hyphae colorless, much narrower than those of subiculum, thin-walled. Cystidia numerous, more concentrated in aculei, scattered elsewhere, usually projecting, arising from subiculum, tubular, sometimes slightly constricted near apices, colorless, 40-200 x 5-8 µm, thick-walled (0.5-2.5 µm thick) except for the thin-walled apex. Basidia suburniform, 7-13 x 3.3-4 µm, 4-sterigmate. Basidiospores cylindrical, adaxially slightly concave, smooth, thin-walled, 4.7-5.5 x 1.8-2.2 µm, IKI-, CB-.

Distribution: Taiwan.

Holotype. Taiwan. Taipei: Kunliao, alt. 100 m, on branch of *Pinus* sp., *leg. Y.F. Lin*, 25 Jul 1991, *Lin 591* (TNM F18973).

Remarks. This new species is similar to *Hyphodontia microspora* J. Erikss. & Hjortstam, but has longer basidiospores. The spore measurements for *H. microspora* were respectively given as 2.5-3.5 x 1.5-1.8 µm (Eriksson & Ryvarden 1976), 3.8-4.5 x 1.8-2.2 µm (Wu 1990), 3.7-4.3 x 1.8-2.1 µm (holotype, Wu 1990) and 2.5-4.5 x 1.5-2.5 µm (Langer 1994). The feature of tubular and thick-walled cystidia in *H. tubuliformis* corresponds with the concept of the *H. barba-jovis* group in *Hyphodontia* (Eriksson & Ryvarden 1976), also equivalent to the generic concept of *Kneiffiella* proposed by Hjortstam & Ryvarden (2002). The author accepts a broader view of the generic delimitation of *Hyphodontia*, treating the new taxon in this genus.

Acknowledgements

This study was supported by the National Science Council of ROC (No. NSC 94-2621-B-178-002). Mr. Y.F. Lin provided the specimen for this study. The author thanks Dr. Nils Hallenberg and Dr. Peter Roberts for reviewing this paper.

Literature Cited

Binder M, Hibbett DS, Larsson K-H, Larsson E, Langer E, Langer G. 2005. The phylogenetic distribution of resupinate forms across the major clades of mushroom-forming fungi (Homobasidiomycetes). Systematics and Biodiversity 3: 113-157.

Eriksson J, Ryvarden L. 1976. The *Corticiaceae* of North Europe 4. *Hyphodermella-Mycoacia*. pp. 547-886. Fungiflora, Oslo.

Hjortstam K, Ryvarden L. 2002. Studies in tropical coticioid fungi (Basidiomycota, Aphyllophorales) *Alutaceodontia*, *Botryodontia*, *Hyphodontia* s.s. and *Kneiffiella*. Synop. Fung. 15: 7-17.

Langer E. 1994. Die Gattung *Hyphodontia* John Eriksson. Biblioth. Mycol. 154: 1-298.

Larsson K-H, Larsson E, and Kõljalg U. 2004. High phylogenetic diversity among corticioid homobasidiomycetes. Mycol. Res. 108: 983-1002.

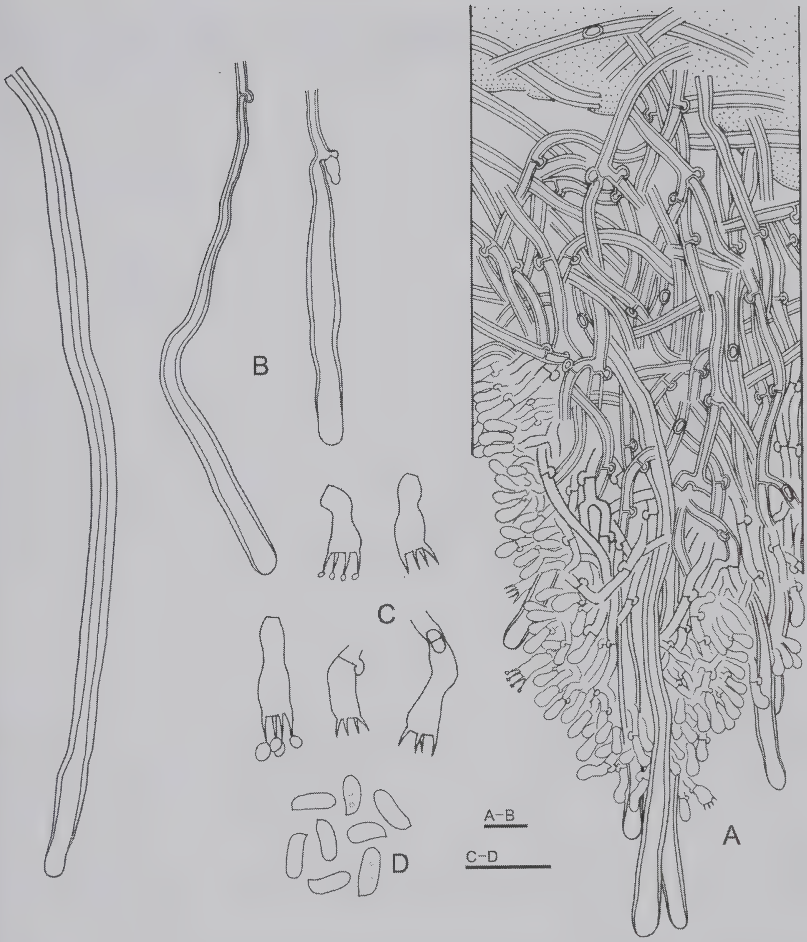


Fig. 1. *Hyphodontia tubuliformis* (holotype). A. Basidiocarp section. B. Cystidia. C. Basidia. D. Basidiospores. Scale bars = 10 μm.

Lin SH, Chen ZC. 1990. The *Corticiaceae* and the resupinate *Hydnaceae* of Taiwan. Taiwania 35: 69-111.

Parmasto E, Nilsson H, Larsson K-H. 2004. Cortbase version 2. Extensive updates of a nomenclatural database for corticioid fungi (Hymenomycetes). Phyloinformatics 1:5

Wu SH. 1990. The *Corticiaceae* (Basidiomycetes) subfamilies *Phlebioideae*, *Phanerochaetoideae* and *Hyphodermoideae* in Taiwan. Acta Bot. Fennica 142: 1-123.

Wu SH. 2000. Studies on *Schizopora flavipora* s.1., with special emphasis on specimens from Taiwan. Mycotaxon 76: 51-66.

Wu SH. 2001. Three new species of *Hyphodontia* with poroid hymenial surface. Mycologia 93: 1019-1025.

MYCOTAXON

Volume 95, pp. 189–194 January–March 2006

Calculating minimum sample sizes for taxonomic measurements: examples using Gäumann's *Peronospora* spore data

ANDREW J. HAMILTON[1,2] & JAMES H. CUNNINGTON[1]

Andrew.Hamilton@deakin.edu.au James.Cunnington@dpi.vic.gov.au

[1]Primary Industries Research Victoria—Knoxfield
Private Bag 15, Ferntree Gully Delivery Centre, Victoria 3156, Australia.

[2]School of Life & Environmental Science, Deakin University
P.O. Box 423, Warrnambool, Victoria 3280, Australia.

Abstract—Methods are presented for calculating minimum sample sizes necessary to obtain precise estimates of fungal spore dimensions. Using previously published spore-length data sets for *Peronospora* species, we demonstrate that 41–71 spores need to be measured to estimate the mean length with a reasonable level of statistical precision and resolution. This is further progressed with examples for calculating the minimum number of spore lengths to measure when matching an undetermined specimen to a known species. Although applied only to spore-length data, all described methods can be applied to any morphometric data that satisfy certain statistical assumptions.

Key word—spore size, spore diameter

Introduction

The question of selecting appropriate sample sizes for taxonomic measurements in mycology is often discussed amongst individuals but rarely raised in the scientific literature. One exception to this has concerned how many spore lengths and widths to measure when undertaking taxonomic studies within the *Peronosporales*, and *Peronospora* in particular (Gustavvson 1959). This arises from Gäumann's (1923) monograph of the genus *Peronospora*, in which he measured approximately 500 spores from each species, believing that 500 measurements were required to produce sufficient data for differentiating similar species.

Using examples from Gäumann's *Peronospora* data, we present methods to address the following two questions:

1. What is the minimum number of spores that need to be measured to determine the mean spore length of a species?
2. What is the minimum number of spores that need to be measured to match an undetermined specimen with a described species?

Methods and Results

Three phylogenetically distinct groups of *Peronospora* species were chosen to infer results that could be applied to *Peronospora* species in general. Gäumann's (1923) data for the species groups on *Vicia* (p. 192), *Stellaria* (p. 48), and *Potentilla* (p. 292) were chosen based on a recent phylogenetic study (Voglmayr 2003) which revealed these to be quite unrelated groups of species. Gäumann used reference numbers to identify the species in his analyses, and the same numbers are used here. The species to which they refer to are: 1 through 4 on *Potentilla* are *P. potentillae anserinae* Gäum., *P. potentillae reptantis* Gäum., *P. potentillae sterilis* Gäum. and *P. potentillae* de Bary; 1 and 2 on *Stellaria* are *P. parva* Gäum. and *P. media* Gäum.; and 1 through 5 on *Vicia* are *P. viciae* (Berk.) Gäum., *P. sepium* Gäum., *P. mayorii* Gäum., *P. viciae sativae* (Thüm.) Syd., and *P. narbonensis* Gäum.. Gäumann's data were manually transcribed from his distribution graphs using a ruler. It is interesting to note that he measured in 1.6 μm units, which is presumably a function of the magnification and the resolution of the graticule he had available. The spore-length ranges (and standard deviations), in μm, were as follows: 8.0–22.4 (2.31), 11.2–25.6 (2.23), 16.0–28.8 (2.15) and 19.2–35.2 (2.50) for species 1 through 4 on *Potentilla*; 9.6–24.0 (2.51) and 19.2–35.2 (3.01) for species 1 and 2 on *Stellaria*; and 9.6–27.2 (2.74), 11.2–30.4 (2.57), 16.0–33.6 (2.51), 19.2–36.8 (2.50) and 22.4–41.6 (2.89) for species 1 through 5 on *Vicia*.

The methods and calculations below were applied to all species in these groups. The methods described here are sensitive to two key assumptions: (i) that the distribution of the measured variable can be closely approximated by the normal distribution, and (ii) that the estimate of the standard deviation, s, of that normal distribution is close to the true standard deviation, σ (Sokal & Rohlf 1969, Zar 1984). Nonetheless, Gäumann's data sets were normal, and morphometric data would generally be expected to be normally distributed.

1. Minimum sample size for estimating the mean spore lengths of *Peronospora* species

The minimum sample size, n, necessary to gain an estimate ($\bar{x}_L$) of the true mean spore-length (μ_L) can be calculated as follows (Harris et al. 1948):

$$n = \frac{s^2 t^2_{\alpha(2),(n-1)} F_{\beta(1),(n-1,\nu)}}{d^2} \qquad \text{equation 1.}$$

where s^2 is the sample variance (assumed to be a good estimate of the true population variance, σ^2), $t_{\alpha(2),(n-1)}$ is the two-tailed critical value for Student's t distribution (with $n-1$ degrees of freedom, *df*), $F_{\beta(1),(n-1,\nu)}$ is the one-tailed critical value for the F distribution (with $n-1$ and ν the numerator and denominator *df* respectively), and d is the half-width of the nominal confidence interval (CI) (i.e. ± distance from $\bar{x}_L$). The confidence level for the confidence interval equals $1-\alpha$ and the assurance that the confidence interval will be no larger than specified is $1-\beta$ ($1-\beta$ = statistical power). An existing data-set is needed to calculate s^2.

A problem arises when trying to solve equation 1, as to obtain $t_{\alpha(2),(n-1)}$ and $F_{\beta(1),(n-1,v)}$ we need to know n, which by definition is unknown. This paradox can be resolved by iteration: solving equation 1 for different values of n until the guess value approximates the value calculated from the equation. The method is perhaps more clearly appreciated in the example below.

Using measurements for Gäumann's 'species 1' on *Vicia* ($n = 424$), we calculated s^2 to be 7.513 (see any standard statistical text to calculate s^2). Suppose we want to be 90% certain that the 95% confidence interval (CI_{95}) is no wider than 1.6 μm (the resolution of Gäumann's measurements), then $d = 0.8$ μm, $1-\beta = 0.90$, $\beta = 0.10$, $1-\alpha = 0.95$, and $\alpha = 0.05$.

We now need to solve for the minimum sample size by iteration. We can start by guessing that $n_{min} = 50$ spores. Thus, $n-1 = 49$ and $\upsilon = 423$, which gives $t_{0.05(2),423} = 2.010$, and $F_{\beta(1),(n-1,\upsilon)} \approx 1.32$. Substituting into equation 1:

$$n = \frac{7.513 \times 2.010^2 \times 1.32}{0.8^2} = 62.58$$

Our guess was less than this estimate so we need to try a higher guess, say 61.

Thus, $n-1 = 60$ and $\upsilon = 423$, which give $t_{0.05(2),423} = 2.000$, and $F_{\beta(1),(n-1,\upsilon)} \approx 1.27$. Substituting into equation 1:

$$n = \frac{7.513 \times 2.000^2 \times 1.27}{0.8^2} = 59.65$$

Therefore, to obtain $\bar{x}_L$ with a 90% probability that the 95% confidence interval will be no wider than 1.6 μm (i.e. ± 0.8 μm), we need to measure more than 59 spores (i.e. 60). Note that there is no point continuing the iteration procedure until the guess and estimate are exactly equal, since fractions of spores will not be counted. Iteration should stop once the smallest integer that is greater than the estimate is reached, keeping in mind that the relationship between the guess and the estimate is dynamic.

We used this procedure to determine the minimum sample size needed to estimate mean spore-length for all the *Peronospora* species considered here. A 1.6 μm CI_{95} was used for all species. The selection of the size of the desired CI_{95} is somewhat subjective. When describing a species for taxonomic purposes a relatively narrow CI_{95} is required, as the estimate $\bar{x}_L$ will be used as a standard by other researchers. We believe that 1.6 μm is sufficiently small to meet this need, and it is also the resolution of Gäumann's measurements.

For the three species groups used in this study, 41 to 71 spores would need to be measured to be 90% certain that the CI_{95} for $\bar{x}_L$ was no wider than 1.6 μm (Table 1). The *Potentilla* species group generally demanded the smaller sample sizes, 41–51, compared to 52–67 for the *Vicia* group, and 52–71 for the *Stellaria* group.

Table 1. The minimum number of spores that need to be measured to be 90% certain that the CI_{95} about $\bar{x}_L$ is no wider than 1.6 μm.

species group (by host plant)	Gäumann's species' reference number 1	2	3	4	5
Potentilla	45	42	41	51	
Stellaria	52	71			
Vicia	60	59	52	59	67

Therefore, inferring from these three phylogenetically distinct groups of species, a conclusion may be drawn that to get an accurate estimate of mean spore length in *Peronospora* species, between about 40 and 70 spores need to be measured.

2. Minimum sample size for estimating the mean spore length to match an unknown specimen with a known species

In the example above we wished to obtain a very precise estimate of μ_L so that this could be used as a reference value. For routine diagnostic examination of specimens, however, such precision is not necessary. Consequently, a wider CI_{95} can be accepted. Once again, the appropriate width of the CI_{95} is somewhat subjective, but will largely depend on the differences between the mean spore-lengths of the species within the group.

This is best illustrated with the two species on *Stellaria* which have mean spore-lengths that differ by 11.07 μm. Thus, in practice, you would only need to measure enough spores to be within 5 μm of the true mean to indicate to which of these two species an undetermined specimen on *Stellaria* would belong, presuming of course that the unknown was one of these two. This value, however, shrinks to 1.7 μm for the species on *Potentilla*, as the minimum difference between any two means is 3.47 μm. Considering these values, we decided to calculate the minimum number of spores that would need to be measured to determine $\bar{x}_L$ with a CI_{95} of 2 μm and 3 μm for *Potentilla*, and 2 μm, 3 μm, 4 μm, and 5 μm for *Stellaria*. The minimum difference between any two species within the *Vicia* group was 0.75 μm, but most species were much farther apart, with the next smallest difference being 2.78 μm. Thus, we calculated minimum sample sizes to obtain CI_{95}s of 0.70 μm, 2 μm, and 3 μm.

The widening of the CI_{95}s for diagnostic measurements substantially reduced the minimum sample-sizes required for all groups (Tables 2–4). Very large numbers of spores would need to be measured if species 2 and 3 on *Vicia* were to be distinguished with any confidence, as indicated by the values for the 0.7 μm CI_{95} in Table 4. In contrast, only 10 spores needed to be measured to differentiate the two species on *Stellaria*.

Table 2. For species found on *Stellaria*, the minimum number of spores that need to be measured to be 90% certain that the CI_{95} about $\bar{x}_L$ is no wider than specified.

nominal CI_{95} (μm)	Gäumann's species' reference number 1	2
2	36	48
3	19	25
4	12	16
5	10	11

Table 3. For species found on *Potentilla*, the minimum number of spores that need to be measured to be 90% certain that the CI_{95} about $\bar{x}_L$ is no wider than specified.

nominal CI_{95} (μm)	Gäumann's species' reference number 1	2	3	4
2	31	29	27	36
3	16	16	15	19

Table 4. For species found on *Vicia*, the minimum number of spores that need to be measured to be 90% certain that the CI_{95} about $\bar{x}_L$ is no wider than specified.

nominal CI_{95} (μm)	Gäumann's species' reference number 1	2	3	4	5
0.7	281	272	235	270	312
2	41	40	36	39	45
3	21	21	19	21	23

Discussion

Gäumann measured around 500 spores for each of the various *Peronospora* species he described, and for some species he measured around 1,000 spores. This is indeed an admirable effort, but in many cases it would not be practicable to measure so many spores. The general question that arises is how many spores need to be measured in morphometric studies? Other studies of downy mildews have involved measuring 100 (Gustavvson 1959), 200 (Smith 1970), or 500 (Ling & Tai 1945) spores. None of these studies described how they determined the number of spores to be measured. Likewise, Hawksworth (1974) recommended making 50–250 spore measurements for general fungal taxonomic studies, but gave no indication as to how he arrived at this range. Furthermore, claims relating to the 'reliability' of particular methods have not been rigorously justified and do not address type I and II error rates (Gustavsson 1959). Presumably, the sample-sizes used in these studies were based on the intuitive notion that more spores would need to be measured when there is large variability in length among spores. While this concept is indeed correct, the subjective manner in which it has been applied almost certainly explains the broad range of minimum sample-sizes propounded. In this paper, more formal and objective approaches that used s^2 as the measure of variability were presented. Also, the question of how many spores to measure was considered more carefully than has been done in the past, with distinctions being made between taxonomic/descriptive and diagnostic requirements.

Regardless of the specific question being addressed, the findings of our work tend to support the general assertion made by others (Gustavsson 1959), namely, that there is no need to measure 500 or 1,000 spores: much smaller sample sizes will suffice. Fewer spores needed to be measured for diagnostic purposes because, compared to the taxonomic agendum, a relatively relaxed level of precision can be accepted. In situations where two or more species have very similar means, as was the case for the *Vicia* group, large numbers of spores need to be measured to distinguish between species.

The techniques described in this paper can be readily applied to other groups of fungi and other morphometric characteristics, such as width or the ratio of width to length, providing such data satisfy the relevant statistical assumptions (i.e. normality and an accurate estimation of s). Also, note that the methods described here assume that s^2 is an adequate estimate of σ^2. This assumption adds further random variation into the estimated sample sizes, so the results should be interpreted conservatively. Where the source data set is large, as was the case for each species here, then s^2 is likely to be a sound estimate of σ^2. But for small data sets, where s^2 may be a poor estimate of σ^2, particular care should be taken in using and interpreting the methods. Caution is also warranted when considering these methods for species where spore morphology is unstable and dependent on environmental factors (Guarro et al. 1997, Arenal et al. 2004).

Acknowledgments

The authors thank Roger Shivas and Wayne Robinson for their expert review and useful suggestions, which improved the quality and clarity of the manuscript. Helpful observations by Shaun Pennycook and Chris Triggs are also appreciated.

Literature cited

Arenal F, Platas G, Peláez, F. 2004. Variability of spore length in some species of the genus *Preussia (Sporormiella)*. Mycotaxon 89: 137-151.

Gäumann, E. 1923. Beiträge zu einer monograhpie der Gattung *Peronospora* Corda. Beitr. Krypt.-fl. Schweiz 4. Zürich.

Guarro J, Abdullah SK, Gené J, Al-Saadoon, AH. 1997. A new species of *Preussia* from submerged plant debris. Mycol. Res. 101: 305-308.

Gustavsson A. 1959. Studies on Nordic peronosporas: II. General account. Opera Botanica 3: 5-61.

Harris M, Horvitz DG, Mood AM. 1948. On the determination of sample sizes in designing experiments. J. Amer. Statist. Assoc. 43: 391-402.

Hawksworth DL. 1974. Mycologists' Handbook. An introduction to the principles of taxonomy and nomenclature in the fungi and lichens. Surrey, Commonwealth Mycological Institute.

Ling L, Tai MC. 1945. On the specialisation of *Bremia lactucae* on Compositae. Trans. Brit. Mycol. Soc. 28: 16-25.

Smith A. 1970. Biometric studies on conidia of *Peronospora tabacina*. Trans. Brit. Mycol. Soc. 55: 59-66.

Sokal RR, Rohlf FJ. 1969. Biometry: the principles and practice of statistics in biological research. San Francisco, WH Freeman and Company.

Voglmayr H. 2003. Phylogenetic relationships of *Peronospora* and related genera based on nuclear ribosomal ITS sequences. Mycol. Res. 107: 1132-1142.

Zar JH. 1984. Biostatistical analysis (2nd edition). Englewood Cliffs, New Jersey, Prentice-Hall.

MYCOTAXON

Volume 95, pp. 195–200 January–March 2006

Tricholoma borgsjoeënse, a new species from a boreal coniferous forest in Fennoscandia

S. Jacobsson[1], S. Muskos[2] & E. Larsson[1]

stig.jacobsson@botany.gu.se
[1]Dept. of Plant- and Environmental Sciences, Göteborg University
Box 461, SE 405 30 Göteborg, Sweden
[2]Klövervägen 13, SE 864 33 Matfors, Sweden

Abstract—*Tricholoma borgsjoeënse*, a species new to science, is described and illustrated. Its systematic position was investigated by use of ITS and LSU nuclear rDNA sequence data. The phylogenetic analysis indicates that the new species belongs in section *Terrea* and is closely related to the American species *Tricholoma atroviolaceum.*

Key Words—Basidiomycotina, *Agaricales, Tricholomataceae,* taxonomy, phylogeny

Introduction

Tricholoma is a large genus with at least 50 species known in Fennoscandia. All of them are ectomycorrhizal with various forest trees, and several well-known species occur in the boreal coniferous forests. However, in some sections of the genus, morphological differences are small and not always evident. One such section is *Terrea* Konrad & Maubl., which comprises a large number of rather similar taxa. This section is characterized by a dry, brownish or greyish, more or less scaly or tomentose pileus surface.

Since 1988 we have observed a rather striking species of this complex in the province of Medelpad, central Sweden that was not possible to identify. It is characterized by a very dark, tomentose-fibrillose pileus, and grows in old, mossy forests with Norway spruce (*Picea abies*). It soon became obvious that it represented an undescribed species, in spite of its rather striking characters.

As the species is connected to old virgin forests, an environment strongly threatened by modern forestry, it is important to have a name for it. The new species is here described as *Tricholoma borgsjoeënse.*

Materials and methods

The complete internal transcribed spacer region (ITS1, 5.8S, ITS2) and approximately 900 base pairs of LSU nuclear rDNA were sequenced for the designated type of the new species *T. borgsjoeënse* SM05/024, *T. borgsjoeënse* SJ030827, *T. orirubens* Quél., RGC04-053, *T. virgatum* (Fr.) P.Kumm., SJ010927 and *T. apium* Jul.Schäff., EL37-99. The sequenced collections are deposited at Göteborg University Herbarium (GB).

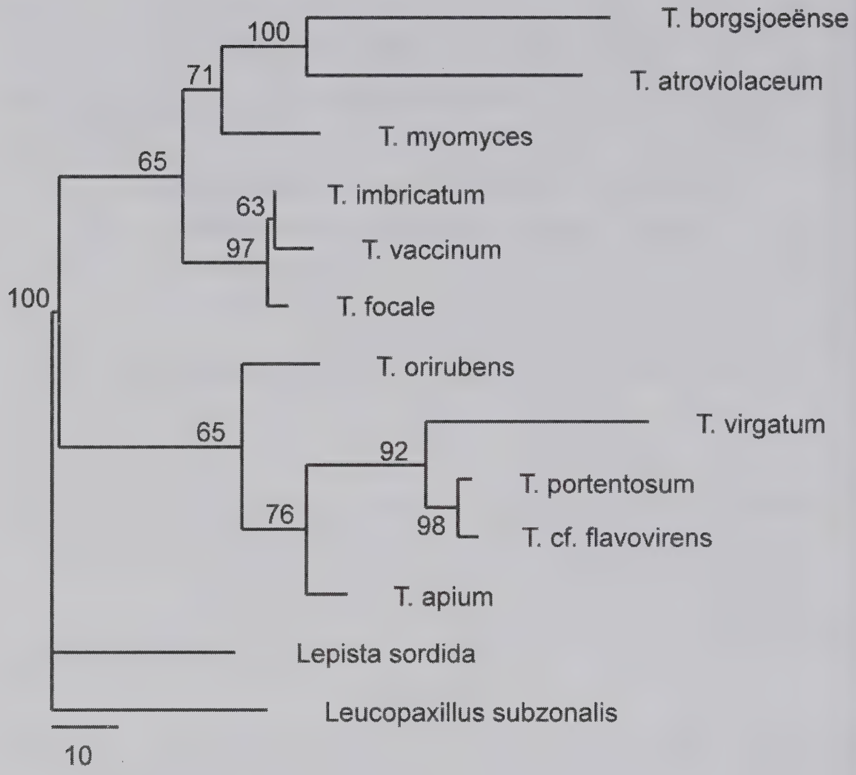

T. borgsjoeënse
T. atroviolaceum
T. myomyces
T. imbricatum
T. vaccinum
T. focale
T. orirubens
T. virgatum
T. portentosum
T. cf. flavovirens
T. apium
Lepista sordida
Leucopaxillus subzonalis
100
71
65
63
97
100
65
92
98
76
10

Fig. 1. The single most parsimonious tree from the phylogenetic analysis, showing the position of *T. borgsjoeënse*. Bootstrap values above 50% are indicated on branches.

Sequences were obtained from fresh material or from herbarium specimens following protocols described in Larsson & Jacobsson 2004. Sequences have been deposited in GenBank (accession numbers DQ415731, DQ389733-DQ389736). Additional sequences were taken from GenBank selected through blast search using the ITS2 region of *T. borgsjoeënse* as target (U7657, AY750166, AF319428, AF18660, U86443, U76458, U76460, U76464, U86672). *Leucopaxillus subzonalis* (Peck) H.E.Bigelow and *Lepista sordida* (Schumach.) Singer were used as the out group.

Sequences were aligned using the data editor in PAUP* (Swofford 2003). Gaps for insertion-deletion events were introduced to aid in the alignment. Heuristic searches for most parsimonious trees were performed using PAUP *. All transformations were considered unordered and equally weighted. Variable regions with ambiguous alignment were excluded, and gaps were treated as missing data. Heuristic searches with 1000 random-addition sequence replicates, TBR branch swapping, were performed. Relative robustness of clades was assessed by the bootstrap method using 1000 heuristic search replicates with 100 random taxon addition sequence replicates and TBR swapping.

Molecular results

The alignment of the 13 specimens was 2198 characters long. After exclusion of ambiguous areas 1623 characters remained for the analysis, of which 1428 were constant, 95 were variable but parsimony uninformative, and 100 were parsimony informative. The maximum parsimony analysis yielded one most parsimonious tree (length=217, CI= 0.7970, RI= 0.6893). Figure 1 shows the single most parsimonious tree presented as a phylogram and bootstrap frequencies are shown on branches. In the tree *T. borgsjoeënse* and *T. atroviolaceum* A.H.Sm. cluster with 100% bootstrap support, indicating a close relationship. The two species further cluster with *T. myomyces* (Pers.) J.E.Lange with a moderate support of 71%.

Taxonomy

Tricholoma borgsjoeënse Jacobsson & Muskos **sp. nov.** FIGURE 2

Pileus 35-88 mm latus, initio convexus, late planoconvexus vel subumbonatus, siccus, tomentosus vel minute squamulosus, atrobrunneus - cinereobrunneus, margine pallidior. Lamellae emarginatae, leviter latae, cineraceae. Stipes 55-105 mm longus, 10-17 mm crassus, saepe flexus, fibrillus, radicans, basi flavida vel albido. Caro pallide cinerea, in pileo atrocinerea, odore farinoso, sapore leviter amaro. Sporae ellipsoideae, 7.5-9 x 5-6 µm, leviae. Cystidia nulla. Hyphae defibulatae. Crescit in silvis abietis inter muscos.

Holotypus: Sweden, Medelpad Muskos 05-024: in herbarium GB concervatus est.

Additional material studied: Sweden, Medelpad, Borgsjö, Julåsen 2005-08-26, leg. Siw Muskos & Jan-Olof Tedebrand; Muskos 05-024 (Holotype, GB), 2003-08-27 (SJ03/012); Mpd, Attmar, Sörlindsjö, 2003-.09-12 (Muskos 03-009, GB); Mpd, Tuna, Kalberget, 1999-08-23 (Muskos 99-009).

Etymology: After the parish of Borgsjö in the province of Medelpad, where several specimens of the species, including the holotype, have been collected.

Pileus 35-88 mm broad when mature, at first obtusely conical with slightly incurved margin, becoming planoconvex, generally without but sometimes with a blunt umbo, dark greyish brown to blackish brown, with age or in dry condition somewhat paler, especially towards the margin, surface dry, completely covered with a dark, blackish tomentum or somewhat fibrillose scaly. **Lamellae** moderately crowded to somewhat subdistant, 40-60 reaching the stipe, strongly emarginate, rather ventricose, up to 12 mm broad, greyish, the edge often becoming blackish spotted when injured. **Stipe** 55-105 mm long and 10-17 mm wide, almost cylindrical or thickest in the middle part, towards the base often curved and more or less rooting, greyish fibrillose but gradually paler towards base and the base pale yellowish, especially when grown in deep moss. No trace of a cortina seen. **Context** in cap thin towards the margin and dark grey marbled, in the stipe dark grey in the upper part but gradually paler towards the base. Odour more or less farinaceous, at least when freshly cut, taste mild or weakly bitter. **Spores** (7-)7.5–9 x 5-6 µm, Q = 1.4-1.8 (20 spores measured), broadly ellipsoid to ovoid with a distinct hilar appendage, smooth, neither amyloid nor dextrinoid. **Basidia** slenderly clavate, 36–48 x 8-10 µm, 4-spored, without clamp connections.

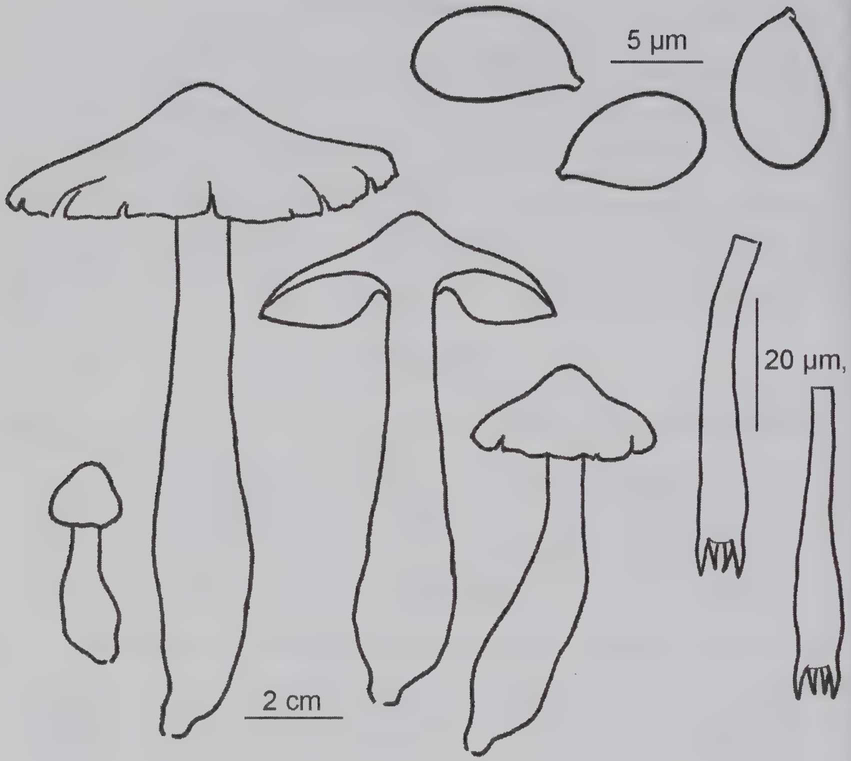


Fig. 2. Spores, basidia and basidiocarps of *Tricholoma borgsjoeënse.*

Lamellae edge fertile, only a few clavate basidioles seen. **Pileipellis** a cutis made up of bundles of strongly septate hyphae, elements 15-30 x 8-20 µm, thick-walled and very dark colored by membranal and sometimes slightly incrusting pigments. Below is a subpellis formed by more inflated and weaker pigmented hyphae, sometimes appearing subcellular. Context of hyphae hyaline and of various thickness. Clamp connections absent in all tissues.

Ecology and distribution—*Tricholoma borgsjoeënse* grows in small groups or rows in boreal coniferous forests of a virgin character, on rather moist and mossy ground with nutrient-rich soil. In all localities Norway spruce (*Picea abies*) was the only or dominant tree, suggesting *T. borgsjoeënse* to form ectomycorrhiza associated with spruce. In this region there is no indication that it could be connected with *Pinus* or any other tree species. Hitherto the species is noted from at least 6 localities in the province of Medelpad, central Sweden.

In the type locality at Julåsen it grows in a rather moist depression not far from a small brook. Within a few meters some other rare agarics have been found as *Hygrophorus purpurascens* (Alb. & Schwein.) Fr. *Cortinarius serarius* Fr., *Limacella glioderma* (Fr.)

Maire. Typical and common species in the same habitat are e.g. *H. erubescens* (Fr.) Fr., *Lactarius scrobiculatus* (Scop.:Fr.) Fr. and *L. badiosanguineus* Kühn. & Romagn. Various ferns and herbs like *Lactuca alpina* are also present. In other localities the habitat is also described as herb-rich spruce forest or in one case more ordinary blue-berry forest. Fruiting time is August - September.

It is surprising that such a striking species has not been described before. To our knowledge it has not yet been found in other parts of Sweden than in the rather small province of Medelpad but it is likely to have a wider distribution. The explanation may be that it is restricted to boreal forests of virgin character. Large areas of boreal forests in the northern half of Sweden are rarely visited by mycologists and much research remains to be done. It is also worth mention that the fruit bodies may often be difficult to discover as a consequence of the dull colors and the growth deep in mosses. The species is found in various parts of Finland according to I. Kytövuori (personal communication), which suggests that it has a wider distribution.

Discussion

Both the macro- and micro-morphological characters of *T. borgsjoeënse* unambiguously indicate that it belongs to section *Terrea*, a section characterized by a dry, woolly-tomentose to squamulose pileus and absence of clamp-connections on the hyphae. Bon (1984) made a subsection *Terreina* containing 13 species in Europe and Riva (1988) included 12 species in the subsection. In Noordeloos & Christensen (1999) only 7 of these taxa were included. Typical members of the subsection *Terreina* are *T. terreum* and its relatives and the complex around *T. orirubens*, a group still poorly investigated and in which the interpretation of some names is disputed.

T. borgsjoeënse clearly differs from the other known taxa belonging to section *Terrea* by several characters: darker colours on pileus and stipe, tomentose rather than a squamulose pileus surface, a fibrous, not squamulose stipe, larger spores and longer basidia. The nature of the pileipellis and pigmentation seems to be rather uniform within the section. Species belonging to the *T. orirubens* group have a strong tendency to redden in parts of the basidiom with age, a character never observed in *T. borgsjoeënse.*

Bresadola (1927, pl. 79) described and depicted a species that shares some characters with *T. borgsjoeënse*, viz. *T. gausapatum* (Fr.)Quél. It possesses large spores (7-9 x 4-4.5 µm), and also the tomentose pileus, somewhat rooting stipe and the occurrence in coniferous forest are characters similar to *T. borgsjoeënse*. Thus *T. gausapatum* ss Bres. may be related to or possibly identical with *T. borgsjoeënse*, but his interpretation deviates in several respects from the original description by Fries (1821). Besides, Fries described *T. gausapatum* as a species growing under deciduous trees in the southernmost part of Sweden.

In the phylogenetic tree *T. borgsjoeënse* cluster with *T. atroviolaceum*, a species described from coniferous forests in western United States (Oregon and California) by Smith (1944). The description shows that *T. atroviolaceum* shares several morphological characters with *T. borgsjoeënse*, e.g. a fibrous pileus surface, large spores and absence of cheilocystidia. However, there are also differences such as the distinct violaceus shade and a subbulbous stipe of *T. atroviolaceum*. No doubt the two species are closely related, but yet distinct from each other.

T. borgsjoeënse and *T. atroviolaceum* form a clade with *T. myomyces*. In contrast, *T. orirubens* and allied species fall in a different clade, in spite of similar morphological characters. However, our dataset includes too few species to be able to infer subgenetic classifications.

Old growth virgin spruce forests are strongly threatened by forestry and most areas with such forest have been clear-cut during the last decades. *T. borgsjoeënse* grows together with species that are on the Red List of Swedish species (Gärdenfors 2005), i.e. *Hygrophorus purpurascens, H. inocybiformis* A.H.Sm. and several species belonging to *Cortinarius* subg. *Phlegmacium*. These species indicate areas of highly valuable undisturbed forest that host many threatened species.

Acknowledgements

We gratefully acknowledge Gro Gulden, Natural History Museum, Oslo, Norway and Henning Knudsen, Botanical Museum, Copenhagen, Denmark for critically reviewing and valuable suggestions to improve the manuscript. Financial support was received from the Swedish species initiative project (ArtDatabanken, SLU).

Literature cited

Bon M. 1984. Tricholomes de France et Eur. occ. 67-278. – Lechevalier, Paris.

Bresadola G. 1927. Iconographia micologica 1, Milan.

Fries E. 1821. Systema Mycologicum. 1. Stockholm.

Gärdenfors U. 2005. The 2005 Redlist of Swedish species. ArtDatabanken, SLU, Sweden.

Larsson E, Jacobsson S. 2004. Controversy over *Hygrophorus cossus* settled using ITS sequence data from 200 year-old type material. Mycological Research 108:781-786.

Noordeloos, M.E., Christensen, M. 1999. *Tricholoma* (Fr.:Fr.) Staude. In Bas, C., Kuyper, Th., Noordeloos, M.E., Vellinga, E. (eds), Flora Agaricina Neerlandica 4:107-152.

Riva, A. 1988. *Tricholoma* (Fr.) Staude. Fungi Europaei 3. Saronno.

Smith A.H. 1944. New North American Agarics. Mycologia 36:242-262.

Swofford D.L. 2003. PAUP*. Phylogenetic Analysis Using Parsimony (* and Other Methods). Version 4. Sinauer Associates, Sunderland, MA.

MYCOTAXON

Volume 95, pp. 201–204 January–March 2006

A new variety of *Meliola thalliformis* from Brazil

Dartanhã J. Soares,
Douglas F. Parreira & Robert W. Barreto

dartjs@yahoo.com.br
Departamento de Fitopatologia, Universidade Federal de Viçosa
Viçosa, Minas Gerais, 36570-00, Brazil

Abstract—*Meliola thalliformis* var. *major* var. nov., associated with leaf spots on *Bathysa australis* (*Rubiaceae*), is described and illustrated.

Key words—tropical fungi, *Meliolales, Meliolaceae,* black mildew

Introduction

During fungal collections undertaken in a stretch of Atlantic tropical rainforest of the nature reserve Parque Estadual da Serra do Brigadeiro in the state of Minas Gerais (Brazil), leaves of the tree *Bathysa australis* (St. Hil.) Benth. & Hook. f. (*Rubiaceae*) were found colonized by a black mildew. Besides the usual black superficial colonies of the fungus, distinct leaf spots were also seen in close association with such colonies.

Bathysa australis is an endemic plant of the Brazilian Atlantic tropical rainforest and it is distributed throughout the South and Southeastern states of Brazil (Germano Filho, 1999). The latter author considered that this species as not threatened with extinction in a broad sense but in the state of Rio Grande do Sul it is considered to be endangered.

A literature survey yielded no fungal records on this host (Viégas, 1961; Minter & Silva, 1995; Mendes et al. 1998; Farr et al. 2005). The occurrence of this fungus represents therefore, the first fungal record on this host which is presented below.

Material and Methods

Freshly collected samples were examined under a stereomicroscope. Hand free sections and adhesive tape slides containing the fungal structures were mounted using lactophenol. Observations, measurements and line drawings were prepared by using an Olympus BX 50 light microscope fitted with a drawing tube. Representative specimens of the fungi were deposited in the herbarium at the Universidade Federal de Viçosa (Herbarium VIC).

Taxonomic Description

Meliola thalliformis var. ***major*** D.J. Soares & R.W. Barreto, **var. nov.**

MycoBank MB500734 (FIGS 1-2)

Differt a var. thalliformis *cellulae myceliales longioris et ascosporis magnioris*

Etymology: named in reference to its larger ascospore size.

Holotype: on *Bathysa australis*, Brazil, state of Minas Gerais, Araponga, Parque Estadual da Serra do Brigadeiro, December 2003, D.J. Soares (VIC-29381).

Colonies on living leaves, predominantly hypophyllous, mostly on the leaf-veins, crustose, to velvety, very densely thalliform, black, seemingly strongly parasitic, directly associated with amphigenous pale brown circular to elliptical leaf spots, 5–40 mm in diam. Hyphae slightly undulate, branching alternate or opposite at acute to wide angle, closely reticulate and thalloid, cells 23–45 x 5.5–11 µm. Appressoria alternate to unilateral, brown to dark brown, straight to slightly curved, 23.5–49 µm long, stalk cells cylindrical, 6.5–23 x 6–9 µm, head cells subglobose to ovate, sometimes angulose, 13–20.5 x 10–16 µm. Phialides separate, on loosely radiating hyphae growing on a upper level above the mycelial plate, opposite or alternate, brown, ampuliform, 14–25 x 7–9.5 µm. Mycelial setae very numerous, both scattered to and grouped around perithecia, mostly widely curved, hooked, rarely substraight, up to 454 x 6–9.5 µm long, dark brown, smooth, tip simple, obtuse, entire, or rounded. Perithecia black, scattered, globose, with crenate to crenulate surfaces, up to 260 µm in diam. Ascospores brown, oblong, obtuse, 4-septate, slightly constricted at septa, 47–61.5 x 12–19.5 µm, smooth.

Comments — 58 specific and 23 infraspecific *Meliola* epithets were included in Hansford's (1961) monograph having members of the family *Rubiaceae* as hosts. None on these fungal taxa had a member of the genus *Bathysa* as a known host. The general morphological characteristics of the fungus described above together with its (uncommon for this group of fungi) evident pathogenic habit agreed well with those described for the species *Meliola thalliformis* Deigthon and *M. thalliformis* var. *naucleae* Deighton. Some biometric differences were nevertheless noted and considered here as sufficient to justify its recognition as a new variety in the species, namely its longer hyphal cells and its larger ascospores. *Meliola canthii* Hansf., another closely-related species having Beeli formulae 3111.5222, is easily distinguishable by the mixed appressoria and phialides and sometimes 2–4-lobate appressoria. Another aspect worthy of consideration is its disjunct distribution as compared with other occurrences of *M. thalliformis* – all recorded only from Africa (Table 1, p. 204).

Acknowledgments

The authors wish to thank the Departamento de Biologia Vegetal of the Universidade Federal de Viçosa for providing support on plant identification, particularly to Michelia Soares, Amilcar Saporetti and Wilson Marcelo. The authors also wish to thank Dr. Bin Song, Dr. Hosagoudar and Dr. Harry Evans for reviewing the manuscript.

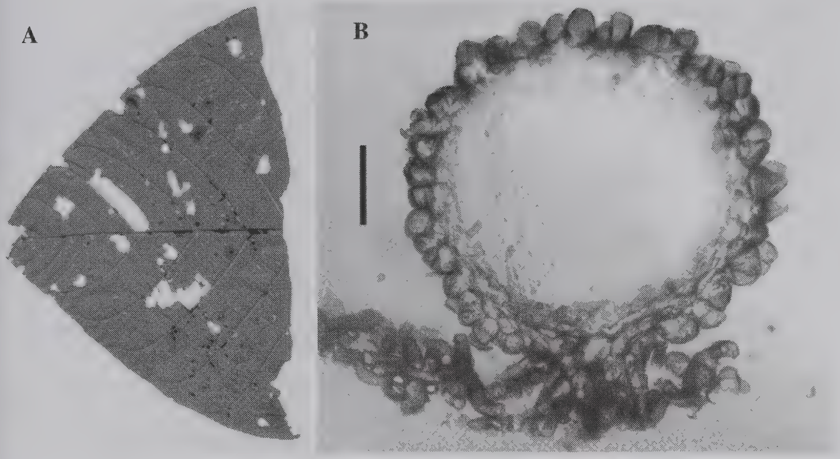


Fig 1. Leaf fragment showing the colonies of *Meliola thalliformis* var. *major* associated with the spots on *Bathysa australis* (A) and transversal section of the perithecium showing the crenulate surface (B). Scale Bar = 30 μm

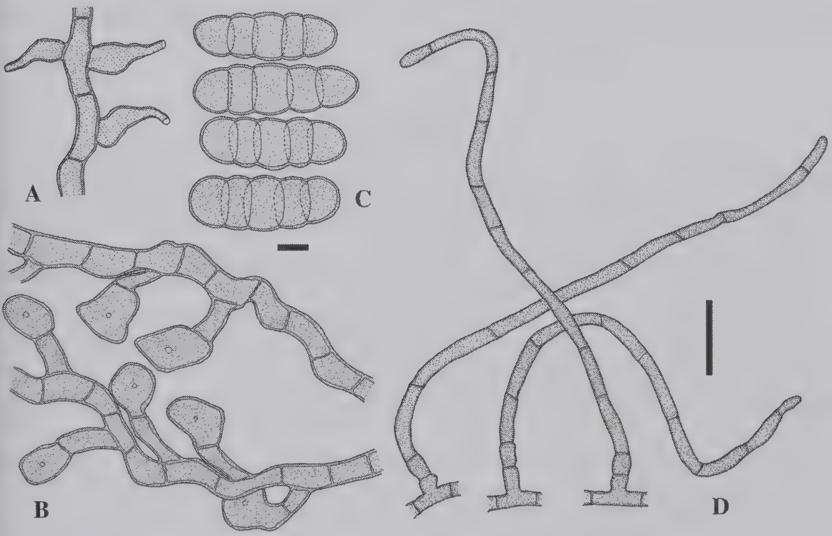


Fig 2. Line drawing of *Meliola thalliformis* var. *major* on *Bathysa australis*. Phialides (A), appressoria (B), ascospores (C) and widely curved and hooked mycelial setae (D). A-C, Scale Bar = 10 μm; D, Scale Bar = 50 μm

Table 1. Morphological features of *Meliola thalliformis* and its varieties.

Features		*M. thalliformis**	*M. thalliformis* var. *naucleae**	*M. thalliformis* var. *major***
Beeli formula		3121.4232	3111.4232	$3121.623^{2}/_{3}$
Hypha cell		10–20 x 6–9 µm	11–20 x 8–10 µm	23–45 x 5.5–11 µm
Mycelial setae		up to 350 x 8–9 µm	up to 320 x 8–9.5 µm	up to 454 x 6–9.5 µm
Appressoria	stalk cell	3–14 µm long	7–12 µm long	6.5–23 x 6–9 µm
	apical cell	11–20 x 10–14 µm	15–22 x 11–16 µm	13–20.5 x 10–16 µm
Phialide		12–20 x 6–8 µm	16–20 x 7–9 µm	14–25 x 7–9.5 µm
Ascoma		up to 230 µm diam	up to 220 µm diam	up to 260 µm diam
Ascospores		38–48 x 14–16 x 12–14 µm	38–48 x 13–15 x 12–13 µm	47–61.5 x 12–19.5 µm
Host		*Mitragyna stipulosa* (DC.) Kuntze	*Nauclea diderrichii* (De Wild.) Merr.	*Bathysa australis*
Geographic range		Sierra Leone	Sierra Leone	Brazil

* Hansford 1961; ** this publication.

Literature Cited

Farr DF, Rossman AY, Palm ME, & McCray EB. 2005. Fungal Databases, Systematic Botany & Mycology Laboratory, ARS, USDA. Retrieved October 12, 2004, from http://nt.ars-grin.gov/fungaldatabases/.

Germano Filho P. 1999. Estudos taxonômicos do gênero *Bathysa* C. Prels. (*Rubiaceae, Rondeletieae*), no Brasil. Rodriguésia 50 (76/77): 49-75.

Hansford CG. 1961. The *Meliolineae* – A monograph. Sydowia Beihefte 2: 1-806.

Mendes MAS, Silva, VL, Dianese JC, Ferreira MASV, Santos CEN, Neto EG, Urben AF, Castro C. 1998. Fungos em plantas no Brasil. Brasília-DF, Embrapa.

Silva M, Minter DW. 1995. Fungi from Brazil recorded by Batista and co-workers. Mycological Papers 169: 585 p.

Viégas AP. 1961. Índice de Fungos da América do Sul. Instituto Agronomico, Campinas-SP, Siqueira.

MYCOTAXON

Volume 95, pp. 205–215 January–March 2006

Russula in Himalaya 2: Four new taxa

KANAD DAS[1*], S.L. MILLER[2]
& J.R. SHARMA[3]

*daskanad@yahoo.co.in
[1]Mycology and Plant Pathology Group, Agharkar Research Institute
G.G. Agarkar Road, Pune 411004, India

[2]Botany Department, University of Wyoming
Laramie, Wyoming 82071 USA

[3]Botanical Survey of India, 192, Kaulagarh Road
Dehradun 248195, India

Abstract—*Russula mayawatiana, R. dhakuriana, R. appendiculata* and *R. puellaris* var. *atrii* are proposed here as new taxa. Phylogenetic positions within the genus *Russula* are supported by macroscopic and microscopic characters as well as rDNA sequences in the ITS gene region.

Key words—*Russulaceae*, taxonomy, ribosomal DNA, phylogeny, India

Introduction

Critical taxonomic studies based on the morphological and anatomical characters of the genus *Russula* from Himalaya have been carried out by different authors (Watling & Gregory 1980, Saini et al. 1982, Atri & Saini 1989, Atri et al. 1991, Atri et al. 1992, Atri et al. 1993, Saini et al. 1993, Atri et al. 1994, Shajahan & Samajpati 1995, Atri et al. 1997, Rawla 2001, Das & Sharma 2003, Das 2005, Das et al. 2006) for the last twenty five years. The flora of the large genus *Russula* from the world over is creating confusion among taxonomists about the proper taxonomic circumscription of species and their placement in the infrageneric classification schemes. Moreover, the morphological and anatomical characters are sometimes not sufficient to comparatively analyse the taxa or establish them as undescribed (new). To eliminate this confusion, efforts have been initiated by a few workers (Miller et al. 2001, Miller & Buyck 2002) to use DNA sequencing to support comparative taxonomic approaches. During a number of macrofungal surveys in Western and North-western Himalaya, the authors gathered a large number of specimens which after thorough morphological and microscopic studies revealed numerous undescribed taxa. Further, DNA sequencing of five taxa also confirmed their status as new to science. Four of them, viz., *R. mayawatiana*, *R. dhakuriana*, *R. appendiculata* and *R. puellaris* var. *atrii* are described and illustrated below, whereas, the fifth one i.e. *R. mukteshwarica* has been submitted (Das et al. 2006) for publication. The molecular tree supporting circumscription of these taxa as new is also provided (Fig. 5, p. 214).

Materials and Methods

Morphological characters were recorded from fresh specimens in the field. Anatomical characterization was done with dry samples by mounting free hand sections of basidiomes in 5% KOH, Melzer's reagent, Congo red, Lactophenol-cotton blue and carbol fuchsin. Colour terms follow Kelly & Judd (1955). Microscopic line drawings were made with the aid of a camera lucida at original magnification of 1500x for basidiospores and 1000x for other microstructures. Density of lamellae (L/cm) was measured at the margin of the pileus excluding lamellulae. Colour codeing used for spore prints is after Romagnesi (1967). Basidiospore length excludes the length of ornamentation. Basidium length excludes the length of sterigmata. Quotient (Q = L/W) was calculated considering the mean value of length and width of 25 basidiospores. Herbarium names used follow Holmgren et al. (1990). Materials and methods for rDNA sequencing were similar to those used in Miller & Henkel (2004).

Description of the species

Russula mayawatiana K. Das, S.L. Mill. & J.R. Sharma **sp. nov.** **Fig. 1.**

Etymology: From Mayawati, referring to the type locality.

Pileus 40–65 mm diam., planoconvexus ad infundibuliformis, crustosus in centro, rufus ad rutilus. Lamellae subdecurrentes, densae, luteae. Stipes 40–50 x 7–12 mm, cylindricus ad subclavatus, luteoalbus. Sporae in cumulo luteae, 7.7–11.5 x 6.2– 9 μm, globosae vel ellipsoidae, amyloideae, verrucosae. Pleurocystidia 68–125 x 9–15 μm, fusiformia. Cheilocystidia 46–70 x 6–9 μm, fusiformia. Pileocystidia cylindrica.

Holotypus: *INDIA, Uttaranchal, Champawat, Mayawati, September 2002, leg. K. Das & J.R. Sharma, KD4542 (**HOLOTYPUS**, BSD; ISOTYPUS, TUR-A).*

Pileus 40–65 mm diam., convex, then planoconvex to infundibuliform with a depression at maturity; pileipellis viscid when moist, often crustose (cracked) at the center, peeling only at margin, deep yellowish pink to medium or deep red or yellowish red, medium to dark or orange yellow at center; margin slightly sulcate. **Lamellae** adnexed to subdecurrent, close (7–8 per cm), forked, pale to orange yellow. **Stipe** 40–50 x 7–12 mm, central, cylindric to subclavate, yellowish white. $FeSO_4$ (+). **Taste** acrid. **Spore print** pale yellow.

Basidiospores 7.7–11.5 x 6.2–9 μm, globose, subglobose, broadly ellipsoid to ellipsoid (Q = 1.05–1.4); ornamentation amyloid, composed of numerous conic warts, up to 1.75 μm high, rarely connected by fine ridges. **Basidia** 40–50 x 7–9 μm, subclavate to clavate, 4-spored; sterigma up to 6 μm long. **Pleurocystidia** 65–125 x 7.7–15 μm, emergent up to 40 μm, abundant, fusiform or with acute, acuminate to narrowly moniliform apex; contents dense. Lamellae edge sterile with few cystidia. **Cheilocystidia** 46–70 x 6–9 μm, fusiform; contents dense. Subhymenium layer up to 20 μm thick, cellular. **Pileipellis** up to 100 μm thick, composed of erect to suberect hyphae and abundant pileocystidia; pileocystidia up to 12 μm, broad, fusiform to cylindrical or acuminate-rostrate, 3–6 septate. **Stipitipellis** composed of mostly repent hyphae and abundant cystidia. **Caulocystidia** up to 8 μm broad, clavate, subclavate or fusoid; contents dense.

Ecology—*Russula mayawatiana* grows in close association with species of *Quercus* and *Rhododendron* in moist mixed temperate forests.

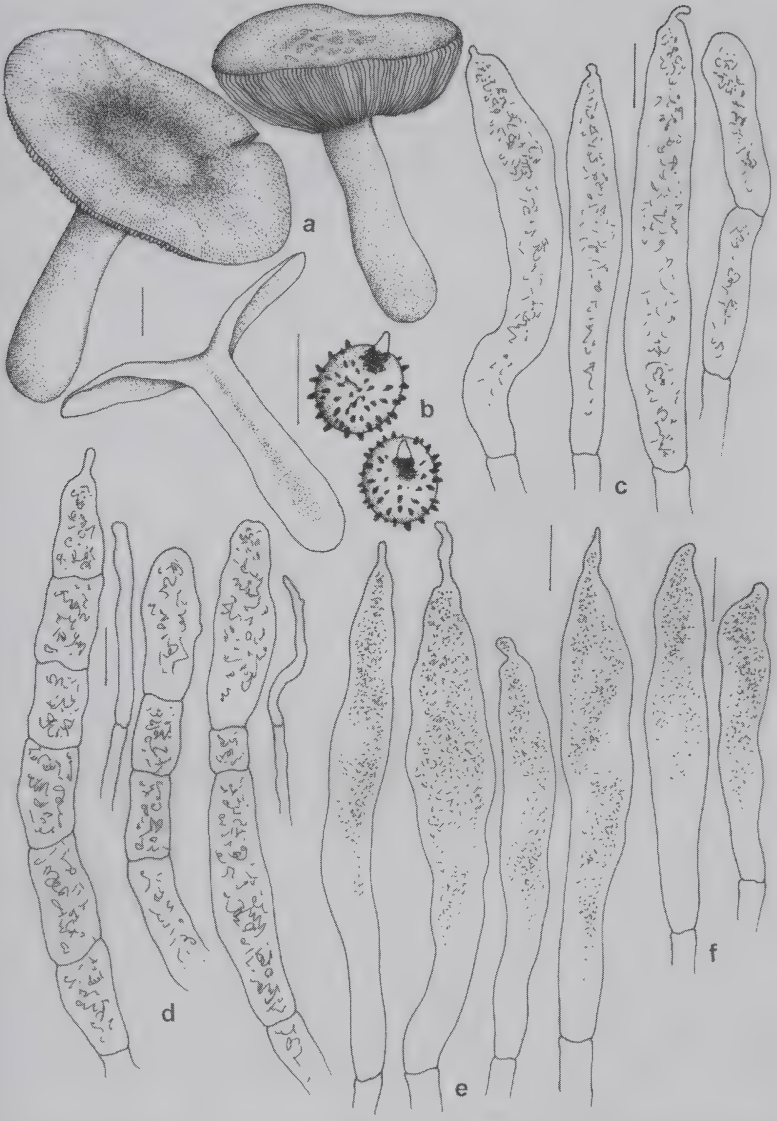


Fig. 1. *Russula mayawatiana* (from **Holotype**): a. Basidiomes b. Basidiospores c. Caulocystidia d. Elements from pileipellis e. Pleurocystidia f. Cheilocystidia. Bars: a = 10 mm; b–f = 10 µm.

OTHER SPECIMENS EXAMINED—INDIA, **Utaaranchal** NAINITAL, Gagar, August 2002, leg. K. Das, KD2149, KD2158 (BSD).

Notes: Bright reddish coloration of pileus, pale yellow spore print and multiseptate (3-6 septate) pileocystidia firmly place *R. mayawatiana* in the subgenus *Russula* emend.

Sarnari. It is differentiated easily by its reddish pileus with yellowish center, distinctly acrid taste, large basidiospores. The present taxon is close to *R. maculata* Quélet & Roze. However, the presence of rusty spotted pileus and stipe surface, non-sulcate pileus margin, non-septate pileocystidia in *R. maculata* separate it from *R. mayawatiana*. Molecular analysis (fig. 5) clearly shows the closeness between these two taxa. Moreover, Rayner (1970) reported spore size ranging from 8–10 x 7–9 µm with wart size up to 1.25–1.5 µm in *R. maculata* as compared to the spore size (7.7–11.5 x 6.2–8.3 µm with warts measuring up to 1.75 µm) recorded for *R. mayawatiana*.

Russula dhakuriana K. Das, J.R. Sharma & S.L. Mill. **sp. nov.** **Fig. 2.**

Etymology: From Dhakuri, referring to the type locality.

Pileus 80–120 mm diam., planoconvexus, leviter depressus in centro, rufus. Lamellae annexae ad subdecurrentes, densae, luteae. Stipes 50–125 x 20–28 mm, subclavatus ad clavatus, roseus. Sporae 6.5–10.2 x 6 –7.7 µm, globosae vel late ellipsoidae, amyloideae, verrucosae. Pleurocystidia 60–115 x 7–12 µm, cylindrica. Cheilocystidia 60–75 x 9–13 µm,clavata ad fusiformia. Pileocystidia absentia

Holotypus: *INDIA, Uttaranchal, Bageshwar, Dhakuri, September 2003, leg. K. Das & J.R. Sharma, KD7029 (**HOLOTYPUS**, BSD; ISOTYPUS, GUH).*

Pileus 80–120 mm diam., convex, planoconvex with depressed center to uplifted at maturity; pileipellis dry, somewhat pruinose but never velvety or scurfy, soft to medium to deep red, often with brilliant orange yellow areas; margin often tuberculately sulcate, split. **Lamellae** adnexed to subdecurrent close to rather crowded (*ca* 8 per cm), often forked from the stipe, brilliant to light orange yellow; lamellulae absent. **Stipe** 50–125 x 20–28 mm, central, subclavate to clavate, yellowish with deep pink to medium red areas, white with tinge of deep pink, $FeSO_4$ (+); context pale yellow. Dried material (lamellae) never red with sulfovanillin. Odour indistinct. **Taste** mild. **Spore print** not obtained.

Basidiospores 6.5–10.2 x 6–7.7 µm, globose to broadly ellipsoid (Q = 1.03–1.26); ornamentation amyloid, composed of isolated conic to spiny warts (up to 1.75 µm). **Basidia** 40–60 x 9–14 µm, subclavate, 4-spored; sterigmata up to 7.5 µm long. **Pleurocystidia** 60–115 x 7–12 µm, emergent up to 45 µm, abundant, cylindrical to subfusoid, thick walled; wall up to 1.5 µm thick; contents dense. Lamellae edge fertile with basidia and cystidia. **Cheilocystidia** 60–75 x 9–13 µm, clavate to fusoid, thick walled; wall up to 1.75 µm thick. **Subpellis** cellular. **Pileipellis** composed of erect to suberect hyphae; hyphae up to 5 µm broad, often with incrustations. **Pileocystidia** absent. Pilear trama with abundant sphaerocytes. **Stipitipellis** composed of suberect to repent hyphae and cystidia.

Ecology—*Russula dhakuriana* grows in close association with species of *Rhododendron* in moist mixed temperate forests.

OTHER SPECIMENS EXAMINED—INDIA, **Utaaranchal** BAGESHWAR, Dhakuri, September 2003, leg. K. Das & J.R. Sharma, KD7092, KD7093 (BSD).

Notes: Morphologically, the bright coloration of pileipellis and microscopically, the presence of incrusted hyphae and absence of pileocystidia in the pileipellis, undoubtedly place these specimens under the subgenus *Incrustatula*. The characters recorded for these

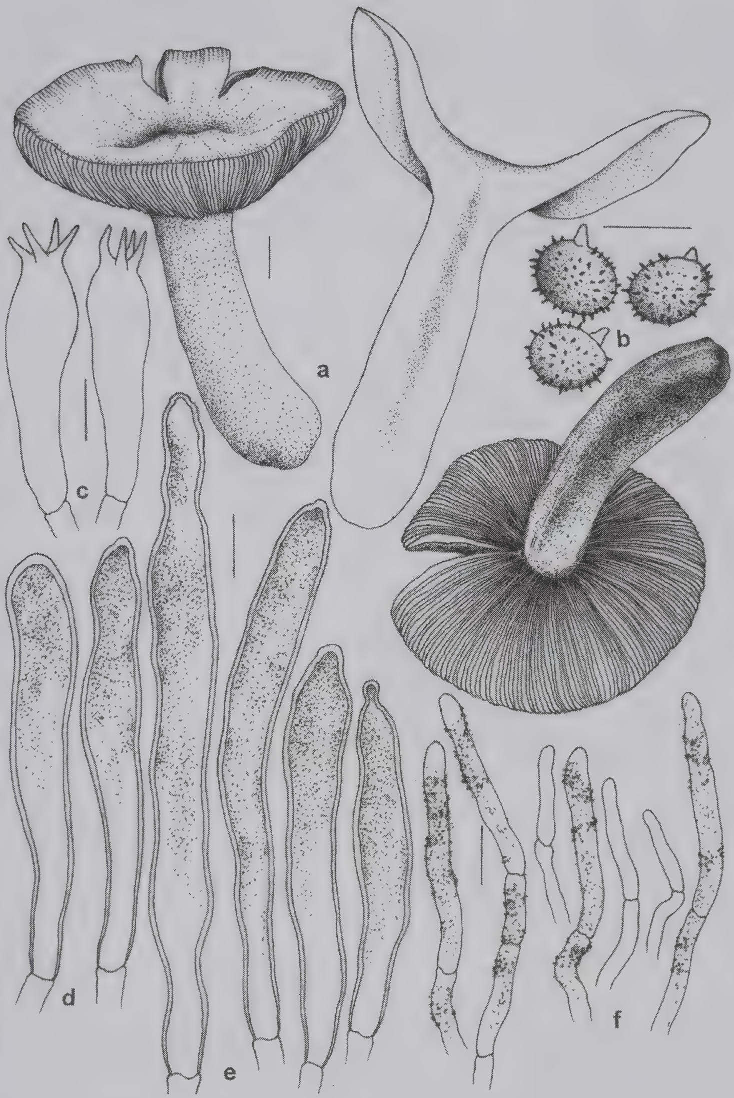


Fig. 2. *Russula dhakuriana* (from **Holotype**): a. Basidiomes b. Basidiospores c. Basidia d. Cheilocystidia e. Pleurocystidia f. Elements from pileipellis. Bars: a = 10 mm; b–f = 10 μm.

specimens are quite close to those of *Russula rosea* Quél., as has also been confirmed by the molecular analysis (Fig. 5). However, the smaller spores and cellular nature of the subpellis are features enough to separate *R. rosea* from *R. dhakuriana.* Further, unlike the present species, the dried lamellae of *R. rosea* stain carmine red in sulfovanillin.

Russula appendiculata K. Das, S.L. Mill. & J.R. Sharma **sp. nov.** **Fig. 3.**

Etymology: After the characteristic appendiculate pleurocystidia.

Pileus 60–90 mm diam., planoconvexus ad infundibuliformis, brunneolus. Lamellae late annexae, luteae. Stipes 37–70 x 13–18 mm, cylindricus ad subclavatus, luteoalbus. Sporae (6) 7–8.8 x 5.4–7.8 µm, globosae vel late ellipsoidae, amyloideae, verrucosae. Pleurocystidia 60–100 x 7.5–12 µm, fusiformia, appendiculata. Cheilocystidia 50–70 x 8–11 µm, cylindrical ad clavata. Pileocystidia cylindrical ad clavata.

Holotypus: *INDIA, Uttaranchal, Champawat, Mayawati,September 2002, leg. K. Das & J.R. Sharma, KD4541 (**HOLOTYPUS**, BSD; ISOTYPUS, TUR-A).*

Pileus 60–90 mm diam., convex, planoconvex to umbelliform with depressed center; pileipellis gelatinous when young, dry at maturity, cracked to areolate towards margin, brownish pink, light gray brown or gray yellowish brown with cream to pale yellow at the center, gray yellow, medium yellow, dark yellow or dark grayish yellow at maturity, never darkening with KOH; margin plane, nonstriate. **Lamellae** broadly adnexed, close (7–8 per cm), forked near the stipe or from the middle, pale yellow; lamellulae in two rows. **Stipe** 37–70 x 13–18 mm, central, cylindric to subclavate, yellowish white, $FeSO_4$ (+); context stuffed, white unchanging. **Taste** mild. **Odor** fruity. **Spore print** not obtained.

Basidiospores (6) 7–8.8 x 5.4–7.8 µm, globose to broadly ellipsoid [Q = 0.95–1.2 (1.30)]; ornamentation amyloid, 0.2–0.4 µm high, composed of irregular isolated warts, few connected by isolated lines, never forming a reticulum. **Basidia** 38–50 x 8–12 µm, clavate, 4-spored. **Pleurocystidia** 60–100 x 7.5–12 µm, cylindric, fusoid or ventricose with rounded, mucronate, appendiculate or narrowly lageniform (tailed) apex, emergent up to 40 µm. **Cheilocystidia** 50–70 x 8–11 µm, cylindrical to clavate. **Pileipellis** composed of suberect septate branched hyphae and cylindrical to clavate pileocystidia with rounded to mucronate apices.

Ecology—Rare, grows in ectomycorrhizal association with species of *Pinus* in mixed temperate forests.

OTHER SPECIMENS EXAMINED—INDIA, Utaaranchal, NAINITAL, Gagar, August 2002, leg. K. Das, KD2176 (BSD).

Notes: Based on the color of the pileus and nature of the pileipellis, the present taxon appears to belong to the subgenus *Heterophyllidia* (Sect. *Rigidae* sensu Singer, 1986) along with *R. virescens* (Schaeff.) Fr. However, the latter has a distinct pseudoparenchymatous subpellis, absence of appendiculate (tailed) pleurocystidia and larger spore ornamentation which clearly separate *R. virescens* from *R. appendiculata.* The absence of any color change of pileipellis with KOH points towards inclusion of *R. appendiculata* under the subgenus *Ingratula* Romagn. emend Sarnari alongwith *R. farinipes* Romell as also supported strongly by molecular analysis (Fig. 5). However, *R. farinipes* differs by having a distinctly nonareolate pileus, larger spore ornamentation and above all lacks the lageniform to tailed pleurocystidia of *R. appendiculata* .

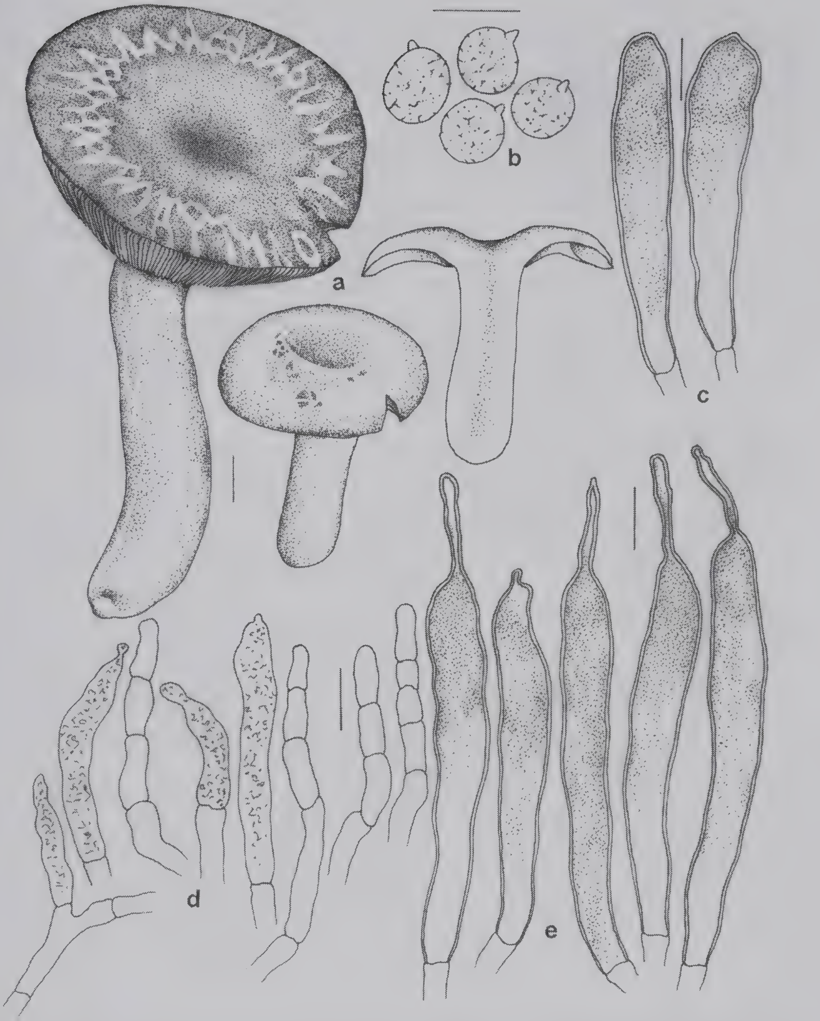


Fig. 3. *Russula appendiculata* (from **Holotype**): a. Basidiomes b. Basidiospores c. Cheilocystidia d. Elements from pileipellis e. Pleurocystidia. Bars: a = 10 mm; b–e = 10 µm.

Russula puellaris var. *atrii* K. Das, S.L. Mill. & J.R. Sharma **var. nov.** **Fig. 4.**

Etymology: In recognition of N.S. Atri for his contribution to the *Russulaceae*.

Pileus 20–35 mm diam., planoconvexus, leviter depressus in centro, atropurpureus Lamellae annexae, luteae. Stipes 23–63 x 5–13 mm, cylindricus ad clavatus, luteoalbus. Sporae in cumulo luteolae, 7.4–9.6 x 6–7.7 µm, subglobosae vel ellipsoidae, amyloideae, verrucosae et corrugatae. Pleurocystidia 50–74 x 9–12 µm, fusiformia. Pileocystidia subclavata ad clavata.

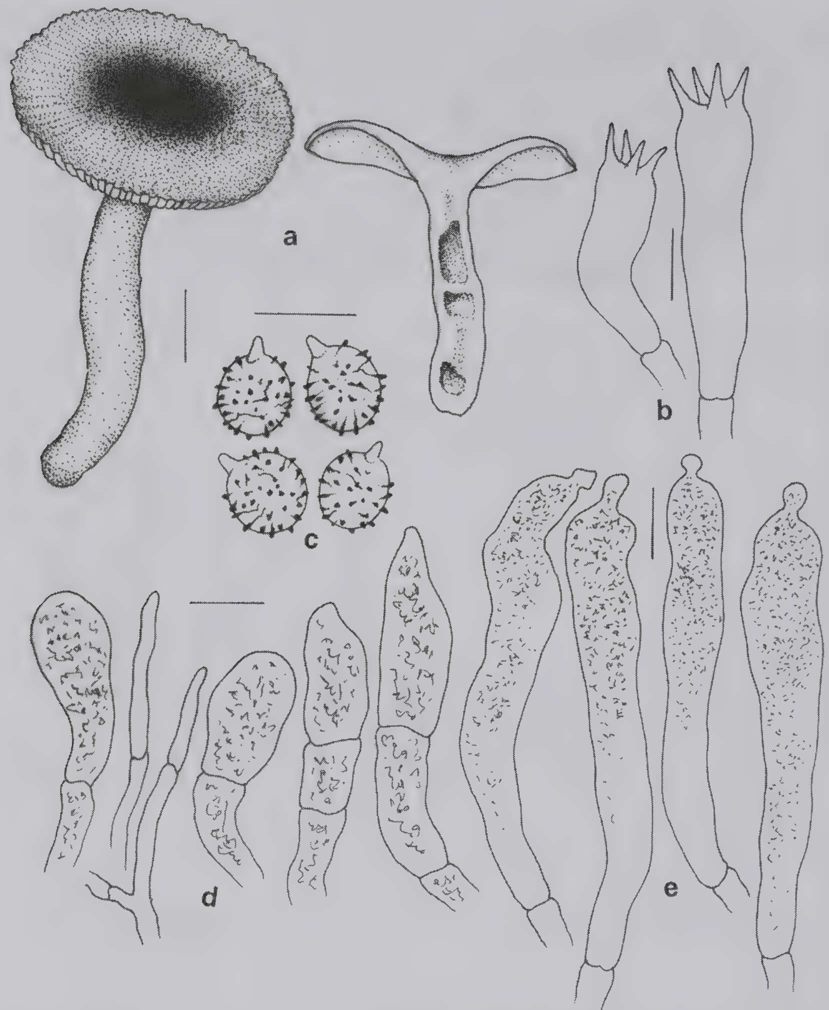


Fig. 4. *Russula puellaris* var. *atrii* (from **Holotype**): a. Basidiomes b. Basidia c. Basidiospores d. Elements from pileipellis e. Pleurocystidia. Bars: a = 10 mm; b–e = 10 μm.

> ***Holotypus:** INDIA, Uttaranchal, Bageshwar, Dhakuri, September 2003, leg. K. Das & J.R. Sharma, KD7022 (**HOLOTYPUS**, BSD).*

Pileus 20–35 mm diam., convex, planoconvex with slightly depressed center at maturity; pileipellis viscid when moist, soft to dark reddish purple with blackish purple center, light grayish or grayish to dark purplish red, slowly yellowish orange at maturity or after bruishing, peeling up to ½ of the radius; margin plane, tuberculately striated. **Lamellae** adnexed to subdecurrent, crowded (10–12 per cm), entire, forked from the stipe, yellowish white, yellowish orange at maturity or after bruising. **Stipe** 23–63 x 5–13 mm, central, cylindrical to clavate, yellowish white, distinctly yellowish orange after bruising or at maturity $FeSO_4$ (+); context white, yellowing after bruising. **Taste** mild. **Spore print** yellowish to buff.

Basidiospores 7.4–9.6 x 6–7.7 µm, subglobose to ellipsoid (Q = 1.08–1.43); ornamentation amyloid, up to 1.4 µm high, composed of conic to spinose warts and ridges, aligned or joined with few connectives to form broken reticulum. **Basidia** 26–45 x 7–11.5 µm, clavate, 4-spored; sterigma up to 6 µm. **Pleurocystidia** 50–74 x 9–12 µm, emergent up to 12 µm, abundant, fusiform to cylindrical with capitate, or appendiculate apices; contents dense. Lamellae edge fertile with basidia and few cystidia. **Cheilocystidia** same as pleurocystidia. Subhymenium layer up to 28 µm thick, cellular. **Pileipellis** composed of upper erect to suberect hyphae up to 5 µm broad and pileocystidia up to 10 µm, broad, subclavate to clavate, 1–2 septate, contents dense; below a layer of parallel compact hyphae.

Ecology—Common, grows in ectomycorrhizal association with species of *Quercus* and *Rhododendron* in temperate deciduous to mixed forests.

> **SPECIMENS EXAMINED** — INDIA, UTTARANCHAL: **Bageshwar**, Dhakuri, September 2003, leg. K. Das & J.R. Sharma, KD7022 (**HOLOTYPE**, BSD); *ibid.*, **Pithoragarh**, Dafia Dhura, October 2001, leg. K. Das & J.R. Sharma, KD4063.

Notes: *Russula puellaris* var. *atrii* is differentiated in the field by the reddish purple pileus with tuberculately striate margin and basidiomes which turn gradually yellow to yellowish orange. The bright coloration of the pileus, septate pileocystidia and nature of hymenial cystidia place the present taxon in the subgenus *Russula*. The morphological and microscopic characters of specimens from Kumaon Himalaya match almost completely those of *R. puellaris* Fr. except slight variation in the spore ornamentation. Romagnesi (1967) reported the spore size ranging from 6.5 – 8.5 (9.5) x 5.5 – 7 µm with wart height extending up to 1.25 µm for *R. puellaris* Fr. var. *puellaris*. Spores are smaller in *R. puellaris* var. *minutalis* (Britzelm.) Singer, 6.5 – 7 (8) x 5 – 6.2 (6.5) µm with warts up to 1.25 µm. The present taxon however, differs from both the above mentioned varieties in having slightly larger spores. The proximity with *R. puellaris* in the parsimonious tree justify the undescribed taxon *R. puellaris* var. *atrii* (Fig. 5).

Acknowledgements

We are thankful to Dr. M. Sanjappa, Director and Dr. D.K. Singh, Joint Director, Botanical Survey of India, Kolkata and Dr. V.S. Rao, Director, Agharkar Research Institute, Pune for providing facilities during the present study, to Dr. N.S. Atri, Punjabi University, Patiala (India) and Dr. Teresa Lebel, Royal Botanical Gardens, Melbourne (Australia) for critically reviewing the manuscript and checking the Latin diagnosis. Editorial assistance by Dr. L.L. Norvell, PNW Mycology Service, Portland (USA) is also duly acknowledged. This research is partially supported by funding to S.L. Miller from the National Science Foundation (DEB – 0315607), USDA (2003 – 01542) and EPSCoR (04 – 47681). We gratefully acknowledge Terry McLean from the Nucleic Acid Exploration Facility at the University of Wyoming for sequencing these specimens.

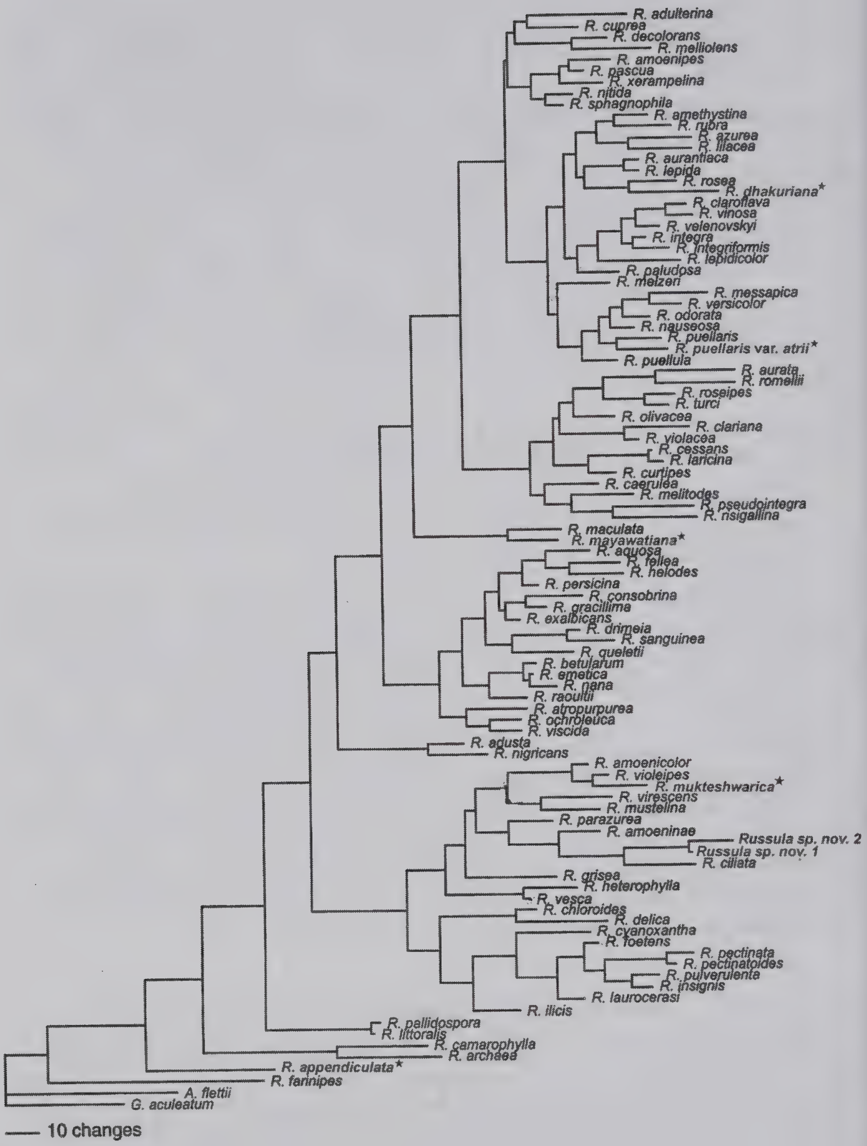

R. adulterina
R. cuprea
R. decolorans
R. melliolens
R. amoenipes
R. pascua
R. xerampelina
R. nitida
R. sphagnophila
R. amethystina
R. rubra
R. azurea
R. lilacea
R. aurantiaca
R. lepida
R. rosea
R. dhakuriana*
R. claroflava
R. vinosa
R. velenovskyi
R. integra
R. integriformis
R. lepidicolor
R. paludosa
R. melzeri
R. messapica
R. versicolor
R. odorata
R. nauseosa
R. puellaris
R. puellaris var. atrii*
R. puellula
R. aurata
R. romellii
R. roseipes
R. turci
R. olivacea
R. clariana
R. violacea
R. cessans
R. laricina
R. curtipes
R. caerulea
R. melitodes
R. pseudointegra
R. risigallina
R. maculata
R. mayawatiana*
R. aquosa
R. fellea
R. helodes
R. persicina
R. consobrina
R. gracillima
R. exalbicans
R. drimeia
R. sanguinea
R. queletii
R. betularum
R. emetica
R. nana
R. raoultii
R. atropurpurea
R. ochroleuca
R. viscida
R. adusta
R. nigricans
R. amoenicolor
R. violeipes
R. mukteshwarica*
R. virescens
R. mustelina
R. parazurea
R. amoeninae
Russula sp. nov. 2
Russula sp. nov. 1
R. ciliata
R. grisea
R. heterophylla
R. vesca
R. chloroides
R. delica
R. cyanoxantha
R. foetens
R. pectinata
R. pectinatoides
R. pulverulenta
R. insignis
R. laurocerasi
R. ilicis
R. pallidospora
R. littoralis
R. camarophylla
R. archaea
R. appendiculata*
R. farinipes
A. flettii
G. aculeatum
10 changes

Fig. 5. Best of 16 most parsimonious trees (1 ln likelihood – 12607, 60827) of 2183 steps inferred from equally weighted parsimony analysis of ITS1, 5.8S and ITS2 rDNA sequences depicted as a phylogram. The CI was 0.285, the RI was 0.530 and the RC was 0.151.

Literature Cited

Atri NS, Saini SS. 1989. Family *Russulaceae* Roze – a review, in M.L. Trivedi, B.S. Gill, S.S. Saini (eds.) 115–128. Plant science research in India, present status and future challenges. New Delhi, India.

Atri NS, Saini SS, Mann DK. 1991. Genus *Russula* Pers. in Dalhousie, in N.C. Aery & B.L. Choudhary (eds.) 92–99. Botanical researches in India. Udaipur, India.

Atri NS, Saini SS, Saini MK. 1993. Some Russulaceous fungi from Dalhousie (H.P.) – The genus Russula Pers. Geobios New Reports 12: 137–140.

Atri NS, Saini MK, Saini SS. 1994. Indian *Russulaceae* Roze – A checklist, in T.A. Sharma, S.S. Saini, M.L. Trivedi & M. Sharma (eds.) 81– 93. Current researches in Plant Science. Dehradun, India.

Atri NS, Saini SS, Saini MK. 1997. Studies on genus *Russula* Pers. from north western Himalayas. Mush. Res. 6 (1): 1–6.

Atri NS, Saini SS, Saini MK, Gupta AK. 1992. Two new records of genus *Russula* from India. Geobios New Reports 11: 101–103.

Das K. 2005. Taxo-Ecological studies on the family *Russulaceae* of Kumaon Himalaya. Text, 179 pp, 31 pl., 75 fig., D. Phil. thesis.

Das K, Sharma JR. 2003. New records of *Russula* from Kumaon Himalaya. Indian Journal of Forestry 26 (3): 320–326.

Das K, Miller SL, Sharma JR, Sharma P, Bhatt RP. (2005) 2006. *Russula* in Himalaya 1: A new species of subgenus *Amoenula*. Mycotaxon 94: 85–88.

Holmgren PK, Holmgren NH, Barnett LC. 1990. Index Herbariorum. Part 1: Herbaria of the world, 86th ed. Brox: New York Botanical Garden.

Kelly KL, Judd DB. 1955. The ISCC-NBS Method of Designating Colors and a Dictionary of Colour Names. ISCC-NBS Color-Name Charts Illustrated with Centroid Colors. National Bureau of Standards Circular 553. U.S. Government Printing Office, Washington, DC.

Miller SL, Buyck B. 2002. Molecular phylogeny of the genus *Russula* in Europe with a comparison of modern infrageneric classifications. Mycol. Res. 106 (3): 259–276.

Miller SL, Henkel TW. 2004. Biology and molecular ecology of subiculate *Lactarius* species from Guyana, in C.L. Cripps (ed.). Fungi in forest ecosystems – systematics, diversity and ecology. New York Botanic Garden, vol. 89, pp 297 – 313.

Miller SL, McClean TM, Walker JF, Buyck B. 2001. A molecular phylogeny of the *Russulales* including agaricoid, gasteroid and pleurotoid taxa. Mycologia 93: 344–354.

Rawla GS. 2001. Himalayan species of *Russula* Pers. Ex S.F. Gray, in P.C. Pande & S.S. Samant (eds.) 1 – 48. Plant Diversity of the Himalaya. India.

Romagnesi H. 1967. Les Russules d'Europe et d'Afrique du nord. Bordas, Paris.

Saini SS, Atri NS, Singer R. 1982. North Indian *Agaricales*–II. Sydowia Annales Mycologici Ser II 35: 238–241.

Saini SS, Atri NS, Anjula, Saini MK. 1993. Studies on the Russulaceous fungi from Narkanda (H.P.) – The genus *Russula* Pers. J. Indian Bot. Soc. (suppl.) p 36.

Shajahan M, Samajpati N. 1995. Ectomycorrhizal fungi of *Shorea robusta* G.f. from West Bengal. Ind. J. Mycol. Res. 33: 105–117.

Singer R. 1986. The *Agaricales* in modern taxonomy. 4th ed. Vaduz J. Cramer. Germany.

Watling R, Gregory NM. 1980. Larger fungi from Kashmir. Nova Hedwigia 26 (3): 320–326.

MYCOTAXON

Volume 95, pp. 217–228 January–March 2006

The distinction between *Menegazzia cincinnata* and *M. valdiviensis* (*Parmeliaceae*)

Mónica T. Adler

adler@bg.fcen.uba.ar
Departamento de Biodiversidad y Biología Experimental
Facultad de Ciencias Exactas y Naturales
Universidad de Buenos Aires (Ciudad Universitaria)
1428 Buenos Aires, Argentina

Susana Calvelo

scalvelo@crub.uncoma.edu.ar
Centro Regional Universitario Bariloche
Universidad Nacional del Comahue
8400 Bariloche RN, Argentina

John A. Elix

John.Elix@anu.edu.au
Department of Chemistry, Faculty of Science
Australian National University, Canberra, ACT 0200, Australia

Abstract—The aim of the present investigation is to assess the status of *Menegazzia cincinnata* and *Menegazzia valdiviensis,* two closely related species from southern South America. The main characters used previously to segregate the two species are discussed and evaluated. The main conclusions are that the presence/absence of thamnolic acid in the thallus medulla correlates well with differences in ascospore length, which, therefore, can be used in combination to propose a new delimitation of both species. In addition, differences in geographic distribution are showed to be consistent with this distinction. A complete chemical profile is detailed for each species, thus redefining, correcting and complementing previously published data. Gyrophoric acid is reported for the first time for the complex and is, with lecanoric acid, consistently present in both species. Other secondary metabolites are reported for the first time for the species.

Key words— Patagonian lichenized Ascomycota, South American lichens.

Introduction

Prior to 1937, *Parmelia cincinnata* was the only known fertile species in subgenus *Menegazzia* Vain. to have a greenish yellow thallus and lacking vegetative diaspores. In 1937 Räsänen described a second species with these features, *Parmelia* (*Menegazzia*) *valdiviensis*, which reportedly differed from the former by its 2-spored asci, in contrast to the mostly 8-spored asci of *M. cincinnata*. The distinction was subsequently maintained by Santesson (1942), who placed *M. cincinnata* in subgenus *Octospora* R. Sant. and *M. valdiviensis* in subgenus *Dispora* R. Sant. However, he used a second key character to distinguish the two species; namely that the apothecial margin of *M. cincinnata* was thick crenulate whereas *M. valdiviensis* had an entire, thin apothecial margin. At that time, cortical and medullary chemistry were not used as taxonomic characters. Calvelo & Adler (1994) and Bernasconi et al. (2002) subsequently utilized the same features to distinguish the two species, because, at that time, the type material of *M. valdiviensis* was unavailable. However, they did note a further possible distinguishing character, the presence of medullary thamnolic acid in *M. valdiviensis* that was absent in *M. cincinnata*. When the type material of *M. valdiviensis* was examined, it became evident that the asci were not 2-spored but 8-spored, or rarely 6-spored as recently published (Bjerke 2005). Consequently, the reported distinguishing characters were reduced to "thalline exciples of young apothecia crenulate and slightly corrugate, lobes distinctly yellow or yellow-green, asci well-developed" for *M. valdiviensis* and "thalline exciples of young apothecia thin, smooth and not crenulate; lobes greenish or yellow-green; asci often underdeveloped" for *M. valdiviensis* (Bjerke 2005). Given these characters, the distinction of the two species is very difficult and they are therefore considered to comprise a complex of two very closely related species.

The present investigation of *M. cincinnata* and *M. valdiviensis* is part of a more extensive study of *Menegazzia* A. Massal. from southern South America (Adler & Calvelo 1996; Calvelo & Adler 1994; Bernasconi et al. 2002). This paper discusses the taxonomic characters used to segregate *M. cincinnata* from *M. valdiviensis*, reports further characters previously overlooked, and proposes a new delimitation of, and redescribes, both species.

Materials and Methods

Collection sites: Samples of *Menegazzia* collected from the western areas of Chubut, Neuquén, Río Negro and Tierra del Fuego provinces between 39° S and 55° S (Argentina), from Chile near the border with Argentina between 36° S and 55° S, and from the southern Chilean Pacific islands were studied. Phytogeographically, the area is quite homogeneous; it belongs to the Antarctic Region, Sub-Antarctic Province (Cabrera 1994). Autochthonous species of *Nothofagus* occur as the principal forest cover, from 700 m to 1,800 m above sea level in the north, and from sea level to 600 m in the south. However, there are important mean annual precipitation differences, varying from 3,000 mm in the north to 545 mm in the southernmost part of the study area (Conti 1998, Tuhkanen 1992).

Specimens examined: The studied material had the main following characteristics: thallus with a grayish yellow, yellowish green to yellowish olive-green upper surface, absence of vegetative propagules, frequently with apothecia, and presence of gyrophoric and lecanoric acids as constant medullary substances. The study is based on *Menegazzia* specimens collected by Adler and Calvelo and on herbarium material. The specimens cited below were selected from a much larger number of collections studied and housed in the following herbaria: BAFC, BCRU, H, S, TUR. Part of the authors' personal collections are kept in the Calvelo private herbarium (and cited as #). An "isolectotype" of *Parmelia cincinnata* (S), previously labeled by Galloway "Cotypus ex herb. Acharius", was examined. A photograph of this specimen, collected by Archibald Menzies in February 1787, at New Year´s Harbour, Staten Island, is available online (http://linnaeus.nrm.se/botany/kbo/ach/ps/L1196.html.en). This specimen had been previously cited as the type material (Santesson 1942). An isotype of *Parmelia valdivienis*, collected by Hollermayer (number 1146 H, ex herb. Räsänen) at Cordillera Pelada, La Reina (Chile) was also studied. So far it has not been possible to clarify whether this specimen is a duplicate of that cited in the protologue (number 6883 of herbarium Gunkel and presumably kept at VALD). We consider that the envelope at H labeled as TYPE (Hollermayer 1146) is the holotype and that a second specimen, lacking the type annotation, is an isotype. The latter was examined during the present study and its identity as an isotype was confirmed by Dr. Orvo Vitikainen (pers. comm.). The names of the herbaria are abbreviated according to Holmgren & Holmgren (2001) and the names of the authors according to Brummitt & Powell (1992).

Laboratory analysis: Thallus and apothecial morphology was examined with a Zeiss Stemi SR dissecting microscope. Observations with the light microscope were performed on hand made thin sections, after immersing the material in 10 % KOH for 1 minute, washed with distilled water, and stained with lactophenol cotton blue. The largest mature ascospores in each slide were chosen and measured to obtain the final size range. For length and width the medial dimensions and standard deviation were calculated. Ascospores measurements were then expressed as minimum value measured – medial minus standard deviation – medial plus standard deviation – maximum value.

Chemical Analysis: The identification of lichen substances present in each of the specimens examined was performed by routine thin–layer chromatography (Culberson & Ammann 1979) and by comparison with authentic samples. The methodology used for high performance liquid chromatography has been described previously (Elix et al. 2003).

Results and Discussion

Species characters in the *Menegazzia cincinnata–M. valdiviensis* complex

During our study of the *Menegazzia* collections, it became obvious that some characters commonly used to segregate *M. cincinnata* from *M. valdiviensis* were ambiguous because intermediate forms were observed (e.g. the apothecial margin) and that the color of the upper surface of freshly collected material was extremely variable. As the frequency of intermediate forms often made it impossible to identify a significant proportion of collections, we were compelled to review the taxonomic value of the characters used previously. These characters are discussed and their use evaluated for consistently separating the two species.

Thallus morphology

Lobe width. The lobe widths reported for *M. cincinnata* were 1.5–2 mm (Santesson (1942), 1.5 mm (Calvelo & Adler 1994), 1.2–2.0 mm (Bjerke 2005) whereas for *M.*

valdiviensis they were 1.5–3.0 (Santesson 1942, Bjerke 2005) and 1–2 mm (Calvelo & Adler 1994). Results from the present study indicate that the range of lobe width throughout the complex is 0.5–2 (–3) mm. Differences observed in lobe width do not correlate with the main differential characters observed in this study, and are therefore not used to segregate the two species.

Branching pattern of the lobes. Previously this has been described as being irregular for *M. cincinnata* (Santesson 1942, Calvelo & Adler 1994, Bjerke 2005), whereas for *M. valdiviensis* it was characterized as being "irregularly dichotomously ramified" (Santesson 1942), dichotomous at the periphery and subdichotomous to irregular at the centre of the thallus (Calvelo & Adler 1994) and irregular (Bjerke 2005). Our present observations show that most of the specimens had subdichotomously branched lobes, more rarely dichotomously branched, and very rarely irregular branched lobes. As a consequence this feature cannot be used to distinguish the two species.

Perforations. For *M. cincinnata* the perforations have been described as rather numerous, small, usually less than 0.5 mm diam. (Santesson 1942), dispersed (sparse) 0.2–0.6 (–1.0) mm diam. (Calvelo & Adler 1994), scarce to numerous (Bernasconi et al. 2002), sparse to numerous 0.2–0.7 (–1.0) mm (Bjerke 2005) whereas for *M. valdiviensis* they were described as sparse and up to 0.7 mm diam. (Santesson 1942), sparse and up to 0.5 mm diam. (Calvelo & Adler 1994) and sparse to numerous, small 0.05–0.7 mm diam. (Bjerke 2005). Our present observations showed that the perforations exhibit similar variation all over the whole complex. The smaller perforations, located at the periphery of the thallus, are 0.05–0.8 mm diam., whereas the larger ones, situated at the thallus centre, are 1–2 (–3) mm diam. Perforations are sparse, moderate or numerous depending on the specimen and are not correlated with other characters. Consequently this feature cannot be used to distinguish the two species.

Color of the upper surface. The upper surface of *M. cincinnata* has been described as pale yellow or yellowish gray, sometimes greenish (Santesson 1942, Calvelo & Adler 1994) to greenish gray (Calvelo & Adler 1994), yellowish gray (Bernasconi *et al.* 2002) or pale yellow or yellowish gray, often with a greenish tinge (Bjerke 2005) whereas the upper surface of *M. valdiviensis* was described as pale grayish yellow or greenish straw–colored (Santesson, 1942), yellowish green (Calvelo & Adler 1994, Bernasconi et al. 2002), greenish gray or pale yellow green (Bjerke 2005). Only Bjerke (2005) segregated the two species using the color of the lobes as a differential character in his key: *M. cincinnata* characterized by "lobes distinctly yellow or yellow–green (paler in shade forms)" and *M. valdiviensis* by "lobes greenish or yellow–green". Our survey of numerous collections has shown this to be an ambiguous character, since numerous intermediate colors can be observed in the field, some even having an olive tinge and other specimens from the north seem to be more yellowish. Further difficulties arise because the color varies between fresh to dry specimens, making it impossible to compare herbarium material, and type specimens in particular. Moreover, the chemical analysis of specimens with different colored upper surface showed no correlating differences in cortical substances or in their concentrations. The variations are probably due to environmental factors yet to be established. For these reasons we consider that the color of the upper surface cannot be used to segregate the two species.

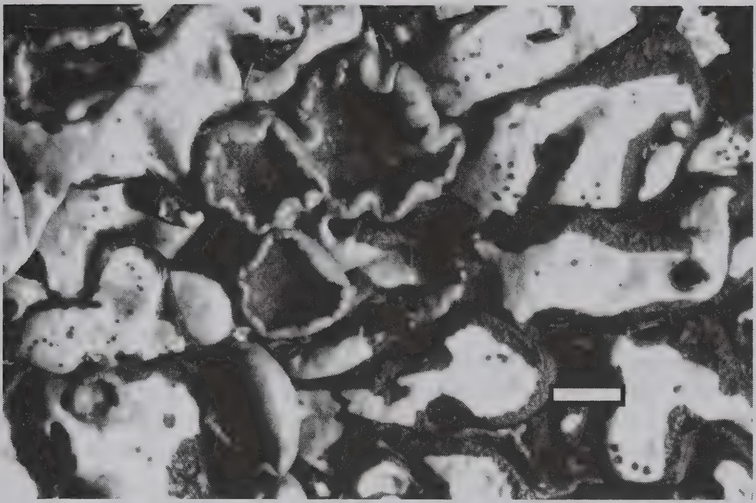


Fig. 1. Part of the isotype of *Parmelia* (*Menegazzia*) *valdiviensis* studied (Hollermayer 1146), showing apothecia with slight to very crenulate (almost incised) apothecial margins.

Scale = 1 mm.

Apothecial morphology and anatomy

Morphology of the Apothecial Margin. The presence of a crenulated apothecial margin was previously considered to be characteristic of *M. cincinnata* (Santesson 1942, Calvelo & Adler 1994, Bernasconi et al. 2002, Bjerke 2005), whereas a thin, entire (smooth) apothecial margin was considered typical of *M. valdiviensis* (Santesson 1942). Even very recently when it was recognized that "the thickness and crenulation of the thalline exciple of *M. cincinnata* is more variable than previously expected" the species were still differentiated as follows: "thalline exciples of young apothecia crenulate and slightly corrugate: *M. cincinnata*" and "thalline exciples of young apothecia thin, smooth and not crenulated: *M. valdiviensis*" (Bjerke 2005). During the present survey, we frequently observed that only one type of apothecial margin (crenulate or entire) commonly develop in a particular specimen, but it was possible to find specimens with both types of apothecia developing side by side, or other thalli with apothecia with slightly crenulate margins among other apothecia with markedly crenulate margin, as is the case for the type of *M. valdiviensis* (Hollermayer 1146, Fig. 1).

A study of numerous collections led us to conclude that this too is an ambiguous character that does not clearly segregate the species. Nevertheless, there is some correlation between the type of apothecial margin and the main differential characters established in the present study, and this will be discussed below (under Relevant taxonomic characters).

Ascospores. The type material of *M. valdiviensis* was recently reported to have mostly 8–spored asci (Bjerke 2005) so it does not belong to subgenus *Dispora* as was previously reported. We have now confirmed that all specimens in the complex generally have asci with 8, sometimes with 6, or rarely with 4 ascospores, and very frequently have mature apothecia with mature asci. Bjerke (2005) reported that *M. valdiviensis* was characterized by the "frequency of underdeveloped asci" but we do not consider this to be a reliable distinguishing character. According to our observations the mature ascospores in all the specimens of the complex are hyaline, ellipsoidal and with smooth to granular inner contents. Very mature (probably over mature) ascospores retained (not released) in the asci are commonly brown to tan. The epispore is commonly rather thick (2–6 µm) in mature ascospores, but is sometimes thinner (1–1.5 µm). The granulation of the inner content, the color of the ascospores and the thickness of the epispore vary in a similar way over all the specimens of the complex, and therefore cannot be used as differential characters.

However, the measurement of a very large number of ascospores revealed the presence of two discontinuous groups of specimens:

Group A, with small ascospores, mostly less than 30 µm in length and in the range [(16-)20–29(-30) × (11-)12.5–20(-22) µm]; similar to that given for *M. cincinnata* by other authors, Santesson (1942) [20–30(-35) × 12–20(-23) µm], Bjerke (2005) [19–28 × 12–18 µm].

Group B, with large ascospores, very frequently exceeding 30 µm in length, and in the range [(20-)26.5–35(-39) × (12-)15–23.5(-26) µm]; similar to that given for *M. valdiviensis* by Santesson (1942): 21–34 × 11–19 µm, but not by Bjerke (2005): 19.0–29.5 ×11.0–18.0 µm.

The possibility of segregating the collections in the complex by ascospores sizes into two groups, and the coincidence of these groups with the two main chemical groups, indicated that ascospores size is a good discriminator, which will be discussed below.

Conidiomata and conidia

In this complex, conidiomata (pycnidia) are frequently present on the thalli immersed in the upper surface. They are globose, 100–200 µm diam. and with a reddish to brown ostiole. Pycnidia characteristics have not been used previously to distinguish *M. cincinnata* and *M. valdiviensis*. We also observed that the conidia are similar throughout the complex, being bacilliform frequently with acute ends to very weakly fusiform, (4-)6–7(-8) µm long. These observations are consistent with previous reports (Santesson 1942, Calvelo & Adler 1994, Bernasconi et al. 2002) and can not be used to segregate the species.

Secondary Chemistry

Thallus chemistry. Previous studies reported that usnic and lecanoric acids were the characteristic substances present in *M. cincinnata* (Calvelo & Adler 1994; Bernasconi et al. 2002; Bjerke 2005). Two additional unidentified compounds were also reported: unid. 1 (absent to minor), with UV spectrum similar to lecanoric acid, and unid 2 (trace to minor), also probably similar to lecanoric acid; thamnolic acid was reported as an accessory substance in some specimens (Bjerke 2005). Usnic, lecanoric and thamnolic acids have been reported as being characteristic for *M. valdiviensis* (Calvelo & Adler 1994, Bernasconi et al. 2002), but Bjerke (2005) reported the presence of usnic and lecanoric acids as constant (characteristic) compounds, together with three other secondary metabolites: thamnolic acid (absent to major), and the unidentified compounds unid. 1

(absent to trace) and unid. 2 (minor to major), the same two compounds reported for *M. cincinnata*.

Our thallus TLC analyses (confirmed by HPLC) of the specimens in the complex led to the identification of the following metabolites: usnic, lecanoric and thamnolic acids (all reported previously), gyrophoric acid, 5–*O*–methylhiascic acid, decarboxythamnolic acid, atranorin, hiascic acid, strepsilin, di–*O*–methylstrepsilin, alectosarmentosin, norascomatic acid and secalonic acid A. More particularly, we established that the medullary depsides gyrophoric and lecanoric acids were present in all specimens (albeit in variable amounts). The third depside, 5–*O*–methylhiascic acid, was detected in the large majority of specimens studied. Our second find concerned the occurrence of thamnolic acid. Approximately half of the collections studied lacked thamnolic acid whereas the remainder contained minor to major amounts of this compound. The specimens could be segregated into two groups: Group A (chemistry): thamnolic acid absent (including the type of *M. cincinnata*) and Group B (chemistry): thamnolic acid present (including the type of *M. valdiviensis*).

Alectosarmentosin and norascomatic acid were detected in some specimens of Group A whereas secalonic acid A was only detected in some specimens of Group B which had yellow patches in the medulla. Traces of atranorin, hiascic acid, strepsilin and di–*O*–methylstrepsilin were detected in some of the specimens in both groups A and B.

In summary, the related depsides gyrophoric, lecanoric, 5–*O*–methylhiascic and hiascic acids are common to all specimens in this complex, so the most important species diagnostic compounds are the β-orcinol *meta*-depsides, thamnolic and decarboxythamnolic acids.

Usnic acid was present in minor to submajor amounts in c. 70% of the specimens of *M. cincinnata*, and c. 50% of the specimens of *M. valdiviensis* as delimited here. In the remaining specimens of both species, usnic acid was present in trace amounts or it was not detected (by TLC). Given this variation, the concentration of cortical usnic acid cannot be used as a differential character in this species complex.

Apothecial chemistry. We observed that the apothecial medullary layer under the hymenium was white except for very old herbarium collections, which frequently have a yellow to orange medulla. Recently the yellow–orange color of the apothecial medulla in this complex was attributed to the presence of anthraquinones (Bjerke 2005). In our investigations the HPLC analysis of apothecia detached from recently collected specimens could not detect anthraquinones and showed that apothecial chemistry was identical to that of the corresponding thallus, apart from the concentration of usnic acid (which was lower in the apothecia).

Ecology and Substratum

The majority of the specimens examined in the present study were collected in *Nothofagus* forests between sea level and c. 1300 m. The specimens were usually corticolous growing on the branches and/or trunks of various species of *Nothofagus* (*N. alpina*, *N. antarctica*, *N. betuloides*, *N. dombeyi*, *N. obliqua*, *N. pumilio*) as well as on *Fitzroya cupressoides*, *Drimys winteri*, and *Lomatia hirsuta*; rarely were they collected on rocks. Neither *M. cincinnata* nor *M. valdiviensis* were substrate specific.

Distribution

In previous studies *M. cincinnata* was reported from Patagonia, Tierra del Fuego and southern Chilean Pacific islands (Santesson 1942), southern South America, particularly for the continental areas of Argentina (Calvelo & Adler 1994), Patagonia and Isla de los Estados, Argentina and Chile (Bernasconi et al. 2002) and southernmost South America from approximately 46° 00' S (Laguna San Rafael) to the southernmost part of Tierra del Fuego (Bjerke 2005). *Menegazzia valdiviensis* was previously reported from the Valdivian region of Chile, Patagonia (area of Nahuel Huapi lake) and from one Pacific Chilean island locality (Santesson 1942, Calvelo & Adler 1994), and Tierra del Fuego (Calvelo & Adler 1994), Patagonia (Argentina and Chile, Bernasconi et al. 2002), southern South America along the Cordillera de los Andes, 37° 40´S (Parque Nacional Nahuelbuta in Chile) to southernmost South America (probably to 52° S, Bjerke 2005).

In the present study we found that the specimens studied corresponding to chemical Group A, thamnolic acid absent, occurred mostly in the Andean and sub Andean localities of Chile and Argentina, 88% at latitudes above 42° S and only 12% in southern Andean localities below 50° S. In contrast, 91% of the examined specimens of chemical Group B, thamnolic acid present, were from southermost localities below 50° S, and only 9% from northern Andean areas of Chile and Argentina.

Relevant taxonomic characters and conclusions

The major result of this study was the discovery that there was a correlation between the presence or absence of thamnolic acid in the thallus medulla and ascospores length. We believe that these two characters should be used to segregate *M. cincinnata* from *M. valdiviensis*. Those specimens with small ascospores (mostly less than 30 µm long) that lack thamnolic acid are considered to represent *M. cincinnata*, whereas those with large ascospores (mostly longer than 30 µm) and with thamnolic acid represent *M. valdiviensis*. Only ca. 10 % of the specimens studied could not be segregated in this manner because they contained thamnolic acid but had small ascospores. This could possibly be explained by the fact that the ascospores were immature, or even that these individuals represent hybrids of the two species.

We also found that *M. cincinnata* and *M. valdiviensis* as segregated here have disjunct distribution in southern South America with the former exhibiting a more northerly distribution (north of 42° S) and the latter with a preference for more southerly areas (below 50° S).

In the case of *M. cincinnata* as defined above, we observed that 80% of specimens had an entire apothecial margin, 8% had a crenulated apothecial margin, and 12% had apothecia with both types of margin. In *M. valdiviensis*, on the other hand, 65% of specimens had apothecia with a crenulated margin, 20% had apothecia with an entire margin and 15% had apothecia with both types of margin. We conclude that the characteristics of the apothecial margin and the color of the thallus can not be used to distinguish the two species because they are too variable. Even so, we did observe that *M. valdiviensis* was more likely to have apothecia with a crenulated margin and *M. cincinnata* more likely to have apothecia with an entire margin, the opposite of what had been accepted previously.

The species

Menegazzia cincinnata (Ach.) Bitter

In Hedwigia 40: 172 (1901).

Parmelia cincinnata Ach., Meth. Lich. 252 (1803). Type: ARGENTINA, Tierra del Fuego Province, Staten Island (Isla de los Estados), New Year´s Harbour, II.1787, A. Menzies, s.n. (E-lectotype fide Galloway 1995, p. 103, S-isolectotype! labeled by Galloway "cotypus").

Lichen cincinnatus Sm. ex Ach., Meth. Lich.: 252 (1803) nom. illeg. Art. 52.1.

Lichen bullatus Menzies ex Ach., Lich. Univ.: 495 (1810) nom. illeg. Art. 52.1.

Parmelia cincinnata Lam., Encycl. Method. Bot., Suppl. 3: 406 (1813) nom. illeg. Art. 52.1.

Thallus foliose, mostly corticolous, up to c. 10 cm diam., tightly attached to the substratum. Lobes narrow, 0.5–2 (–3) mm wide, straight or irregularly zigzagging, parallel and contiguous at the margins, subdichotomously or dichotomously branched or rarely with irregular branching, not parallel at the centre. Upper surface without soredia or isidia, grayish yellow to greenish yellow, sometimes with olive tinge. Perforations circular to elliptical, 0.05–2 (–3) mm diam., sparse or moderate, rarely numerous, always on the median line of the lobes. Medulla white in the upper part of the cavity, black in the lower part. Lower surface black, scrobiculate.

Apothecia common, adnate to shortly stipitate, disc imperforate, 1–4 (–10) mm diam., pale to chestnut brown to dark brown; margin mostly entire, not crenulated, or sometimes slightly crenulated, rarely markedly crenate, sometimes with both types of margin in the same thallus. Asci elongated to subglobose, 100–120 × 50–60 μm when mature, usually with 8, sometimes with 6 or rarely with 4 ascospores. Ascospores hyaline, ellipsoid, smooth to slightly granular (vacuolated) within when mature, with a brown epispore when overmature, (16–) 20–29 (–30) × (11–) 12.5–20 (–22) μm, epispore (1–) 2–3 (–6) μm thick.

Conidiomata (pycnidia) common, immersed, globose, up to 200 μm diam. Conidia bacilliform to weakly fusiform commonly with acute ends, (4.5–) 6–7.5 (–8) × 1–1.5 μm.

Chemistry: Upper surface K–; containing usnic acid (submajor to trace), ±atranorin (trace). Medulla K– or K+ pale yellow, sometimes orange in the algal layer, C– or C+ pink or red, sometimes red in the algal layer, KC– or KC+ pink, orange or red; containing gyrophoric acid (major to minor), lecanoric acid (minor to trace), ±5–*O*–methylhiascic acid (minor to trace), hiascic acid (minor to trace), alectosarmentosin (minor to trace), strepsilin (minor to trace), ±3–*O*–methylstrepsilin (trace), ±di–*O*–methylstrepsilin (trace), ±norascomatic acid (trace).

REPRESENTATIVE SPECIMENS EXAMINED— **Argentina**. Prov. de Chubut. Lago Futalaufquen, E side, on *Nothofagus. antarctica*, 20 I 1938, A. Kalela 238a (H). Prov. del Neuquén. Lago Aluminé, Angostura, c. 1400 m, *Araucaria araucana–N. pumilio* forest, 24 XII 1937, A. Kalela 156d (H); 3 km SE of Angostura, 1120 m in heath, on *N. antarctica*, 24 XII 1937, A. Kalela 165c (H). Paso Tromen, near Río Malleo, on *N. alpina*, 15 II 1994, Messuti s.n. (Herb. Calvelo # 951). Lago Quillén, S side, on *N. obliqua*, 28 XII 1937, A. Kalela 179c (H). Lago Nahuel Huapi, San Leo, alt. 1100 m, on twigs of *Nothofagus*, 1896, P. Dusén 165 (S). Provincia Río Negro. Bariloche, Arroyo Casa de Piedra, on *N. dombeyi*, alt. 800 m, 28 V 1995, Calvelo (# 990), 5 I 1995 Calvelo (# 992); E side, alt. 850 m, on *N. dombeyi*, 7 II 1986, Calvelo (# 57); W side, 16 V 1988, Calvelo (# 749). Cerro López, 1060 m, on *N. pumilio*, 4 IV1987, Calvelo (# 48), 1300 m, 25 V 1999, Calvelo (# 1614). Lago Gallardo, 5 III 1999, Calvelo (# 1618). Llao Llao, pathway Soria Moria to Lago Escondido, on *N. dombeyi*, 7 X 1993, Calvelo (# 913). Cerro Bella Vista, W slope, alt. 1350 m, on *N. pumilio*, 27 I 1990, Calvelo (Herb. C.-422). Cerro Catedral, 1300 m, on *N. pumilio*, 24 III 1987, Calvelo (# 752, BCRU–000754). Cerro Chalhuaco, 31 X 1999, Bernasconi s. n. (# 1617). Cerro Tronador, pathway to Castaño Overa, 1150 m, on *N. pumilio*, 8 IV 1993, Calvelo (# 844), 1300 m, Calvelo (# 845). Puerto Blest, 14 VII 1897, Dusén 162a (S), Puerto Blest, pathway to Laguna Ortiz Basualdo, on *Fitzroya cupressoides*, 1300 m, 12 III 1994, Calvelo (# 954). Villa Tacul, coast of lago Nahuel Huapi, on *Lomatia hirsuta*, 7 X 1993, Calvelo (# 918). Provincia de Tierra del Fuego. Isla de los Estados, Bahía Flinders, W part of bay, 54° 49' S, 64° 36' W, 120 m, on *Nothofagus*, 7 XI 1972, Imshaug 53457 (BCRU–01597). Isla Grande, Aguas Claras, Calvelo & Adler XI 1997 (BAFC–39218). **Chile**. Región de los Lagos, Prov. de Llanquihue. Parque Nacional V. Pérez Rosales, mallín, III 1970, Redón 02157 (TUR–12299).

Menegazzia valdiviensis (Räsänen) R. Sant.

In Ark. Bot. 30A(11):26 (1942).

Parmelia valdiviensis Räsänen, Revista Univ. (Santiago) 22: 197 (1937). Type: CHILE, Provincia de Valdivia, Cordillera Pelada, la Reina, cortícola, 900 m., 30.XI.1934, A. Hollermayer 1146, ex herb. Räsänen (H–holotype, H–isotype!).

Thallus foliose, corticolous or rarely saxicolous, tightly attached, up to c. 10 cm diam., orbicular. Lobes narrow, 0.5–2 (–3) mm wide, straight or irregularly zigzagging, mainly parallel and contiguous, infrequently separated, subdichotomously to dichotomously branched, not parallel at the centre of the thallus. Upper surface lacking isidia or soredia, greenish yellow to grayish yellow, occasionally with olive tinge. Perforations circular to elliptical, 0.1–2 (–3) mm diam., sparse to moderate, rarely numerous, occurring on the median line of the lobes. Medulla white, rarely with yellow patches in the upper part of thallus cavity, black in the lower part. Lower surface black, scrobiculate.

Apothecia adnate to shortly stipitate, disc imperforate, chestnut brown to tan, 1–4 (–5) mm diam., margin usually crenulated, infrequently entire, sometimes apothecia with both types of margin same thallus. Asci elongated to subglobose when mature, 100–150 × 50–70 µm, mostly with 8, sometimes with 6, or rarely with 4 ascospores. Ascospores hyaline, ellipsoid, smooth inside to somewhat granular when mature, with a brown to tan epispore when over mature. Ascospores (20–) 26.5–35 (–39) × (12–) 15–23.5 (–26) µm, epispore (1–) 2–3 (–6) µm thick.

Conidiomata (pycnidia) frequent, globose, immersed, 85–120 µm diam.; conidia bacilliform to weakly fusiform, (5–) 6.5–7.5 (–8) × 1–1.5 µm, commonly with acute ends.

Chemistry: Upper cortex K–; containing usnic acid (submajor to trace), ±atranorin (trace). Medulla K+ yellow, C– or C+ pale pink, KC+ yellow turning reddish orange; containing gyrophoric acid (major to minor), lecanoric acid (minor to trace), ±5-*O*-methylhiascic acid (minor to trace), thamnolic acid (major to minor), decarboxythamnolic acid (minor to trace), hiascic acid (trace), ±secalonic acid A (minor), ±strepsilin (trace), ±di-*O*-methylstrepsilin (trace), ±alectosarmentosin (trace).

REPRESENTATIVE SPECIMENS EXAMINED— **Argentina**. Provincia de Neuquén. Lago Traful, on slope, on *N. pumilio*, 19 XI 1937, A. Kalela 59:b (H). Provincia Río

Negro. Bariloche, Lago Nahuel Huapi, 5 XI 1896, P. Dusén 168 (S). Prov. de Tierra del Fuego. Isla Grande, Cerro Cabras, on *N. pumilio*, 460 m, 28 XII 1986, S. Stenroos 2161 (H), Valle Carbajal, Sierra de Sorondo, *Nothofagus* forest, 54° 43' S, 68° 07' W, 300 m, 28 XI 1971, Imshaug 55564 (BCRU–01683); N slope, on *N. pumilio*, 200 m, 6 II 1940, Santesson 750 (UPS), Cerro Chennen, SE slope, alt. 200–250 m, 54° 21' S, 67° 52' W, *N. pumilio* and *N. antartica* forest, 8 I 1989, Ahti (48163) & Stenroos (H), Río Larsiparsalik, on *N. betuloides* forest, XI 1997, Calvelo & Adler s. n. (BAFC–39172, 39173, 39174, 39175), Laguna Victoria, 70 m, on *N. pumilio*, Calvelo & Adler s. n. (BAFC–39168, 39169, 39170, 39171, 39176). Isla de los Estados, Port Cook, 18 XI 1902, C. Skottsberg s. n., ex Herb. Erik P. Vrang (H), Puerto Parry, *Nothofagus–Drimys*, 54° 47' S, 64° 22' W, near sea level, 10 XI 1971, Imshaug 53906 (BCRU-01598). **Chile**. Región de Magallanes, Prov. de Magallanes, Estrecho de Magallanes, NE side of Pto. Gallant, 53° 41' S, 72° 00' W, 6 X 1969, Imshaug 45104 (BCRU–01675. Isla Clarence. Southern peninsula, 40 m, 16 I 1987, S. Stenroos 2583 (H); 110 m, on *N. betuloides*, 17 I 1987, S. Stenroos 2922b (H). Isla Desolación, Puerto Angosto, 12 IV 1896, P. Dusén s.n. (S); 16 IV 1896, P. Dusén s.n.(S); 12 IV 1896, P. Dusén 226 ex herb. Malme (S). Isla Furia, E part, alt. 100 m, on *Drimys winteri*, 20 I 1987, S. Stenroos 2639 (H); on *N. betuloides*, 40 m, 20 I 1987, Stenroos 2633 (H); 100 m, on *N. betuloides*, 20 I 1987, S. Stenroos 2635 (H). Isla Newton, 30 V 1896, P. Dusén 48 (S). Prov. Última Esperanza, Fjordo de Agostini, 54°26´S, 70°26´W, 22 II 1929, H. Roivainen s. n. (H). Fjordo Almirante Martínez, 52°52'S, 73°28´W, on *N. betuloides*, 21 II 1929, H. Roivainen s.n. (H).

Acknowledgements

We are grateful to the curators of the following herbaria for the loan of material which in some cases included type specimens: BAFC, BCRU, H, S, TUR and to Dr Orvo Vitikainen for the critical information on collections. We thank Dr. Alan Fryday and Dra. Sionara Eliasaro for the revision of the manuscript. The study was supported by Secretaría de Investigación, Universidad Nacional del Comahue (Project B04 122) and CONICET (Consejo Nacional de Investigaciones Científicas y Técnicas, Argentina), Programa de la Flora Criptogámica de Tierra del Fuego and Grant PIP 2268. MTA is Member of the Research Career of CONICET (Argentina).

Literature Cited

Adler MT, Calvelo S. 1996. Two new species of the genus *Menegazzia* (*Parmeliaceae* sensu lato, lichenized Ascomycotina) from Southern South America. Mycotaxon 59: 367–372.

Brummitt R, Powell C. 1992. Authors of Plant Names. Royal Botanical Garden, Kew.

Bernasconi E, Calvelo S, Adler MT. 2002. *Menegazzia* (*Parmeliaceae*: Ascomycota) and an associated lichenicolous fungus, *Abrothallus parmeliarum*, from Patagonia, Argentina. Aust. Syst. Bot. 15: 527–534.

Bjerke JW. 2005. Synopsis of the lichen genus *Menegazzia* (*Parmeliaceae*, Ascomycota) in South America. Mycotaxon 91: 423–454.

Cabrera AL. 1994. Regiones Fitogeográficas Argentinas. Enciclopedia Argentina de Agricultura y Jardinería: Tomo 2, Fasc. 1. 1ra. reimpresión, Acme. Buenos Aires.

Calvelo S, Adler MT. 1994. *Menegazzia* (Ascomycota, Liquenizado) en la Argentina. Bol. Soc. Argent. Bot. 30: 119–125.

Conti HA. 1998. Características climáticas de la Patagonia en Flora Patagónica. Parte 1, Colección Científica del INTA, Buenos Aires, Correa, Director.

Culberson CF, Ammann K. 1979. Standardmethode zur Dünnschichtchromatographie von Flechtensubstanzen. Herzogia 5: 1–24.

Elix JA, Giralt M, Wardlaw JH. 2003. New chloro-depsides from the lichen *Dimelaena radiata*. Biblioth. Lichenol. 86: 1–7.

Galloway DJ. 1995. The extra-European lichen collections of Archibald Menzies MD, FLS (1754-1842). Edinb. J. Bot. 52: 95–139.

Holmgren P, Holmgren N. 2001. Index Herbariorum. Part 1. The Herbaria of the World, ed. 8. Regnum Vegetabile 120. New York Botanical Garden, NY.

Räsänen V. 1937. Líquenes Chilenos coleccionados por el R. P. Atanasio Hollermayer en 1927–36. Rev. Univ. (Santiago). 22: 195–211.

Santesson R. 1942. The South American *Menegazziae*. Ark. Bot. 30A (11):1–35.

Tuhkanen S. 1992. The climate of Tierra del Fuego from a vegetation geographical point of view and its ecoclimatic counterparts elsewhere. Acta Bot. Fennica 145: 1–64.

MYCOTAXON

Volume 95, pp. 229–240 January–March 2006

NATS truffle and truffle-like fungi 13: *Tuber quercicola* and *T. whetstonense*, new species from Oregon, and *T. candidum* redescribed

Jonathan L. Frank[1], Darlene Southworth[1]
& James M. Trappe[2]

frankjon@sou.edu
[1]Department of Biology, Southern Oregon University
1250 Siskiyou Blvd. Ashland, OR 97520-5071, USA

[2]Department of Forest Science, Oregon State University
Corvallis, OR 97331-5752, USA

Abstract—Two new truffle species, *Tuber quercicola* and *T. whetstonense* (*Tuberaceae, Pezizales*), are described from southern Oregon, USA. *Tuber candidum* is redescribed. These hypogeous species are ectomycorrhizal with *Quercus garryana*. Descriptions of *T. whetstonense* and *T. candidum* include ectomycorrhizal morphotypes.

Key words—hypogeous fungi, mycorrhizas, DNA

Introduction

Recent research on biodiversity of ectomycorrhizal fungi in oak woodlands included a survey of hypogeous sporocarps at Whetstone Savanna Preserve in southern Oregon (Valentine et al. 2004, Frank et al. 2004, Frank 2005). The site is a remnant woodland of *Quercus garryana* Dougl. ex Hook. interspersed with *Ceanothus cuneatus* (Hook.) Nutt. shrublands. Eighteen species of hypogeous fungi were collected for this survey (Frank 2005), of which three were in the genus *Tuber: T. candidum, T. quercicola* sp. nov. and *T. whetstonense* sp. nov.

Molecular methods were used to compare field collections to hypogeous fungi in GenBank and to match sporocarps to ectomycorrhizas (Gardes & Bruns 1993, Valentine et al. 2004, Frank et al. 2004). Our molecular analysis supports the proposition that spiny-spored and reticulate-spored *Tuber* species form distinct clades (Zobel 1854, Frank 2005).

Tuber candidum, the most frequently collected hypogeous species at the study site, was also among the more common ectomycorrhizal morphotypes collected, second only in frequency to the ubiquitous *Cenococcum geophilum* Fr. (Valentine et al. 2004, Frank 2005). Spores from all three *Tuber* species were present in the fecal pellets of three small mammals, *Microtus californicus, Peromyscus maniculatus*, and *Reithrodontomys megalotis*, trapped at the research site (Frank 2005).

Materials and Methods

Whetstone Savanna Preserve (42°25'N, 122°54'W), owned by The Nature Conservancy, is on an alluvial plain near the Rogue River in southern Oregon. Precipitation is 40-50 cm of rainfall per year. Hypogeous sporocarps were collected by raking the ground with a short-tined garden cultivator beneath and around *Quercus garryana* (Oregon white oak, Garry oak) weekly from April through June, 2003 through 2005, and less frequently in other months. Leaf litter and soil were examined for sequestrate fruiting bodies. Specimens were described the same day (Weber et al. 1997). Sporocarps were photographed in the field with a Canon EOS digital SLR camera and in the lab under a Leica MZ75 dissecting microscope with a SPOT RT Color camera (Diagnostic Instruments, Inc.) and under a Leica DMLB compound microscope with a SPOT QE insight camera. Microscopic characters were described from razor-blade sections of fresh specimens mounted in 5% aqueous KOH. Sections were stained with Melzer's reagent (Castellano et al. 1989), rinsed and mounted with polyvinyl lactic acid glycerol (Brundrett et al. 1996) to make permanent slides for archiving with dried specimens. Collections were deposited in the herbaria of Southern Oregon University (SOC) and Oregon State University (OSC).

Mycorrhizas were obtained from soil cores taken under mature trees and seedlings (Valentine et al. 2004). Roots were rinsed over sieves, examined and mycorrhizal morphotypes photographed under the dissecting microscope. Ectomycorrhizal outer mantles were peeled with a sharp needle, mounted in water, and photographed under the compound microscope. Description of cell morphology of sporocarps and mycorrhizas follows terminology in Goodman et al. (1996).

DNA was extracted from sporocarps and mycorrhizas with CTAB and amplified in polymerase chain reactions (PCR) with fungal specific primers ITS1F and ITS4 (White et al. 1990, Gardes & Bruns 1993). Molecular data were obtained by sequencing of the internal transcribed spacer (ITS) region, including ITS1, the 5.8S ribosomal DNA gene and ITS2. PCR products were cleaned in Montage PCR Centrifugal Filter Devices (Millipore Corporation). Clean PCR products were prepared for sequencing with BigDye Terminator Ready Reaction Mix and sequenced by an ABI 310 Genetic Analyzer (Applied Biosystems). Sequences were edited with Chromas 1.45 (McCarthy 1998) and compared to other fungal sequences in GenBank, the NCBI electronic database (*www.ncbi.nlm.nih.gov*), with BLAST and to each other with ClustalX (Altschul et al. 1990, Thompson et al. 1997). Sequences were submitted to GenBank; accession numbers are given with the collection numbers under each species description. Phylogenetic alignments were generated and edited in ClustalX. Phylogenetic trees using maximum parsimony and bootstrap values from 1000 replicates with full heuristic searches were generated in PAUP* 4.0b 10 (Swofford 2002). All characters were equally weighted and unordered. Gapped positions were treated as missing data (gap = missing).

Taxonomic Descriptions

Tuber candidum Harkn., Proc. Cal. Acad. Sci. Ser. 3 Bot. 1: 274, 1899.

Figures 1, 2, 4, 5

= *Tuber olivaceum* Harkn., Proc. Cal. Acad. Sci. Ser. 3 Bot. 1: 275, 1899.
= *Terfeziopsis lignaria* Harkn., Proc. Cal. Acad. Sci. Ser. 3 Bot. 1: 279, 1899.

Ascomata globose to irregularly subglobose stereothecia, 1-4 cm x 1-5 cm. **Peridium** smooth, light yellowish brown to reddish brown. **Gleba** firm, hard when dried, light tan to dark reddish brown, intricately marbled with thin white sterile veins continuous with the inner peridium. **Odor** mild to slightly earthy; **taste** bland.

Peridium 100-300 μm thick with two layers: **outer layer** 30-100 μm thick, light brown, of irregularly compact to elongated cells 4-10 x 2-5 μm; **inner layer** 70-200 μm thick, hyaline, of interwoven hyphae with irregularly elongated cells 5-15 x 2-3 μm. **Glebal trama** hyaline, of irregularly isodiametric to elongated cells 4-15 x 2-5 μm, sometimes with interhyphal spaces. **Asci** globose to subglobose, 60-85 x 45-70 μm with a double wall and a stem 15-50 μm long with a forked base, 1-5 spored, hyaline, arranged randomly throughout the glebal fertile tissues.

Spores light brown, subglobose to ellipsoid, 19-42 x 14-34 μm excluding ornamentation and depending on number of spores per ascus: in 1-spored asci 36-42 x 26-34 μm, 2-spored 28-36 x 22-30 μm, 3-spored 23-31 x 18-25 μm, and 4-5-spored 19-30 x 14-25 μm; **ornamentation** of curved spines 2-5 μm long at maturity; **spore walls** 1-2 μm thick.

Habit, habitat and season hypogeous; associated with *Quercus garryana* in southern Oregon, 400 m elevation, Mediterranean climate with dry summers and less than 50 cm annual precipitation; also associated with *Quercus* spp. from Washington and Idaho south to southern California. Sporocarps fruit below leaf litter in top 2-7 cm of mineral soil, March to August in our study area but year round over the entire range.

Ectomycorrhizal morphotype tan to brown with lighter tips, cystidia lacking; **outer mantle** of interlocking irregular synenchyma to net synenchyma; **inner mantle** non-interlocking irregular synenchyma to regular synenchyma.

Collections examined: HOLOTYPE — UNITED STATES. California: Placer Co. Auburn, Harkness 195, May (BPI, isotype OSC). OTHER COLLECTIONS — **UNITED STATES. California: Alameda Co.** Oakland, Mountain View Cemetery, N. Gardner 133, 4 May 1903 (OSC 55902), **Butte Co.** Merrimac, T. Norman, 29 Nov 1933, Trappe 2567 (OSC 80591). **Los Angeles Co.** San Gabriel Mtns., Evey Canyon, 4.5 mi N of Claremont, R. Benjamin, 13 Oct 1955, Trappe 17610 (OSC 80596). **Mariposa Co.** Hwy 120 at Greeley Hill Road, M.A. Castellano, 27 Mar 1985, Trappe 8440 (OSC 46928). **Monterey Co.** Hastings Natural History Reserve, Melin River, J.M. Linsdale, 53, 23 May 1945, (OSC 80617). **Placer Co.** Auburn, H. W. Harkness 197, May (Holotype of *Tuber olivaceum* BPI, Isotype OSC); H. W. Harkness 206, June (Type of *Terfeziopsis lignaria* BPI, Isotype OSC). **Sacramento Co.** Sacramento, N. L. Gardner, undated, Gilkey 1149 (OSC 80615). **San Mateo Co.** Portola Valley, S. Rutherford, 4 May 1975, Trappe 4089 (OSC 80592). **Idaho: Ada Co.** Boise, C. Baker, 1976, Trappe 4908 (OSC). **Oregon: Benton Co.** 4.5 mi. W of Alpine, M. Hinds, 5 July 2004, Trappe 29542 (OSC). **Douglas Co.** Elkton, Phipps Nursery, G. Menser, 9 Apr 1986, Trappe 8879 (OSC). **Jackson Co.**

Medford, Whetstone Savanna Preserve (42°25'N, 122°54'W). J. L. Frank, 30 Apr 2004, 727 (SOC, OSC 111407, GenBank AY830856); 27 May 2003, 500, 501, 502; 13 June 2003, 512; 8 July 2003, 516; 8 August 2003, 520; 17 March 2004, 608; 25 March 2004, 618, 619, 627; 6 April 2004, 657, 661, 663, 673; 15 April 2004, 675, 677, 678, 679, 681, 683; 23 April 2004, 693, 694, 695; 26 April 2004, 698; 27 April 2004, 703, 705, 708, 711, 715, 719, 721; 30 April 2004, 735; 25 May 2004, 739, 740, 743; 31 May 2004, 746, 748, 750, 752, 754 (OSC 111408), 755; 1 June 2004, 757, 758 (OSC 111409), 759 (OSC 111410); 3 June 2004, 768; 5 June 2004, 778; 6 June 2004, 783 (OSC 111411) (SOC). Ashland, Emigrant Lake (42°08'N, 122°36'W). J. L. Frank, 2 April 2004, 645, 5 April 2004, 652 (SOC). **Linn Co.** near Lebanon, L. Taylor, 3 Dec 1959, Gilkey 948 (OSC 80599). **Marion Co.** Turner, Marion Hill, Otis Bynum, 20 June 1980, Trappe 5831 (OSC). **Polk Co.** 5 mi. W. of Monmouth, J. Trappe 1604, 10 Sep 1968 (OSC 80590). **Yamhill Co.** Chehalem Mtn., S.M. Zeller, Gilkey 1144 (OSC 80613).

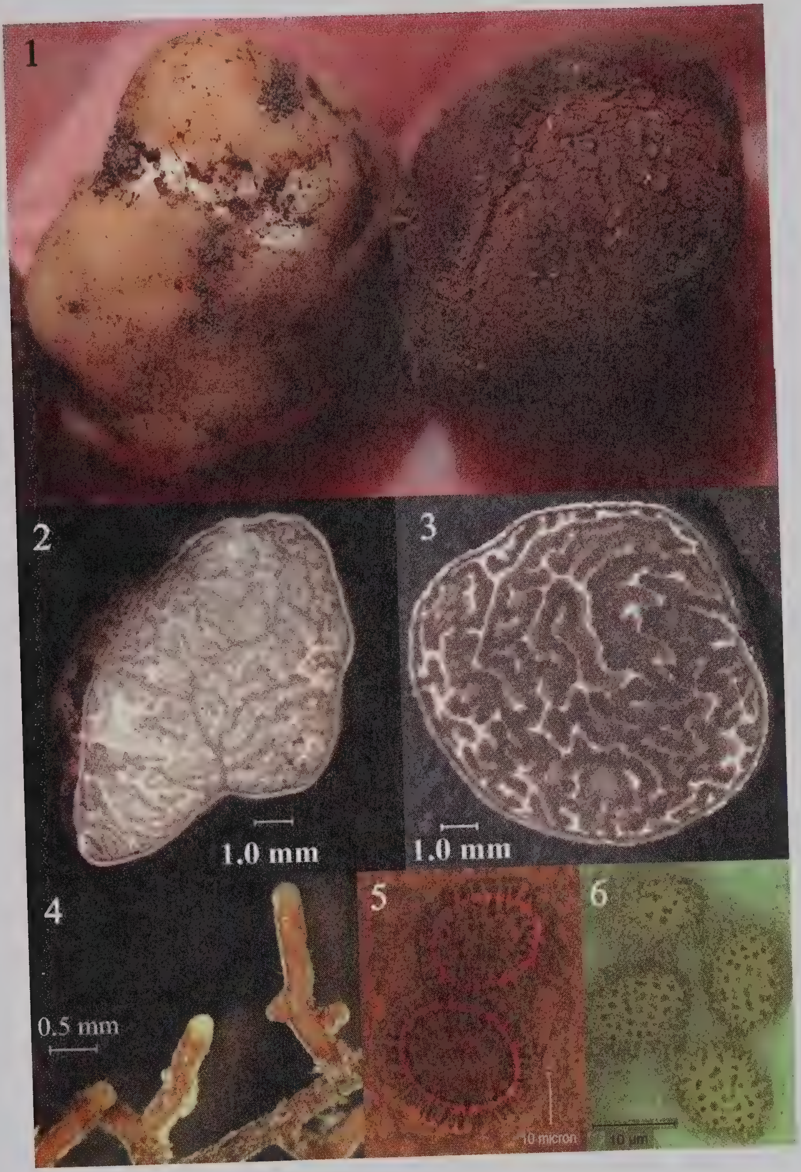
COMMENTS – *Tuber candidum* was given two other names by Harkness (1899): *Tuber olivaceum,* a glebal color variant that is a slightly immature stage of *T. candidum,* and *Terfeziopsis lignaria,* a very immature stage of *T. candidum* with a white gleba. The anatomy of all three is identical except for developmental differences. *T. candidum* is similar enough to the European *T. nitidum* Vittad. that the former was regarded as synonymous with the latter by Lange (1956), Ceruti (1961) and Trappe (1968). Although *T. candidum* and *T. quercicola* (below) are morphologically close to *T. nitidum* and *T. rufum* respectively, DNA sequences indicate that the North American species are distinct from the European species (Figure 7). Additionally, unlike the *T. rufum* group, including forma *nitidum*, forma *ferrugineum*, and variety *rufum*, the North American species *T. candidum* and *T. quercicola* are morphologically and molecularly distinct (Montecchi & Sarasini 2000, Ceruti et al. 2003). *T. candidum* and *T. quercicola* are distinctive among the *Tuber* species analyzed here; both have an 85-90 base pair insert in ITS1. Whereas sequences of *T. rufum* (AF106892) and *T. ferrugineum* (AF132506) are nearly identical, sequences *T. candidum* and *T. quercicola* differ by over 10% in ITS1 and ITS2. Accordingly, despite the lack of a sequence for *T. nitidum,* it seems appropriate to retain the name *T. candidum* for the North American genotype.

The mycorrhizal status of *Tuber candidum* was confirmed by DNA sequences. ITS sequence data from SOC727 (OSC 111407; GenBank AY830856) matched a mycorrhiza (SAP0901; GenBank AY969005) collected from *Quercus garryana* roots at Whetstone Savanna Preserve; the two sequences are greater than 97% similar.

Tuber quercicola J.L. Frank, Southworth & Trappe **sp. nov.** **Figures 1, 3, 6**

Ascomata globosa vel subglobosa, stereothecia, 1-3 cm lata. Peridium 250-500 μm crassum, verruculosum vel squamosumque rimosum, obscure rubrum vel rubiginosum, stratis duobus: exteriore 20-100 μm crasso, hyphis arcte intertextis; interiore 130-400 μm crasso, hyphis laxe vel arcte intertextis. Gleba firma, brunneola vel atroporphyrea, venis angustis albis sterilibus intricate marmorata. Asci irregulariter globosi vel subglobosi, 62-80 x 40-55 μm, parietibus duplicibus, stipite 10-35 μm longa, sporis 1-5. Sporae brunneolae, ellipsoideae, 20-45 x 15-35 μm, spinis 2-5 μm longis ornatae. Typus J. L. Frank 738 (SOC).

Etymology: Latin, *querci-* (oak) and *-cola* (dweller), "dweller with oaks."



Figures 1–6. Comparison between *Tuber candidum* and *T. quercicola* sp. nov. **Figure 1.** External surfaces: *Tuber candidum* (left) vs. *T. quercicola* (right). **Figures 2–3.** Cross-sections. **Figure 2.** *T. candidum* (SOC602). **Figure 3.** *T. quercicola* (SOC747). **Figure 4.** *T. candidum* mycorrhiza (MAT0901). **Figures 5–6.** Ascospores. **Figure 5.** *T. candidum* (SOC512). **Figure 6.** *T. quercicola* (SOC702).

Ascomata globose to subglobose stereothecia, 1-3 cm broad. **Peridium** minutely verrucose to scaly and cracked, dark red to brownish red. **Gleba** firm, hard when dried, light tan to dark reddish brown, intricately marbled with thin white sterile veins continuous with inner peridium. **Odor** mild to slightly earthy or often of fresh green beans. **Taste** bland.

Peridium 250-500 μm with two layers: **outer layer** 20-100 μm thick, rusty brown, of loosely to tightly interwoven hyphae 3-6 μm broad; **inner layer** 130-400 μm thick, of hyaline, loosely to tightly interwoven hyphae 2-4 μm broad. **Glebal trama** of hyaline, tightly interwoven hyphae 3-5 μm broad. **Asci** irregularly globose to subglobose, 62-80 x 40-55 μm, with a double wall and a stem 10-35 μm long and with a forked base, 1-5 spored, hyaline, arranged randomly throughout the glebal fertile tissue.

Spores light brown, broadly to narrowly ellipsoid, 20-45 x 15-35 μm excluding ornamentation and depending on number of spores per ascus: in 1-spored asci 36-45 x 26-35 μm, 2-spored 30-36 x 24-30 μm, 3-spored 22-32 x 20-26 μm, and 4-5-spored 20-28 x 15-20 μm; **ornamentation** of curved spines 2-5 μm long at maturity; **spore walls** 1-2 μm thick.

Habit, Habitat and Season: hypogeous; associated with *Quercus garryana* in southern Oregon, 400 m elevation, Mediterranean climate with dry summers and less than 50 cm annual precipitation; also occurring elsewhere in western Oregon to northern California. Sporocarps fruit below leaf litter in top 2-7 cm of mineral soil, March to August.

> **Collections examined: HOLOTYPE— UNITED STATES. Oregon: Jackson Co.** Medford, Whetstone Savanna Preserve (42°25'N, 122°54'W), J. L. Frank, 25 May 2004, 738 (SOC, isotype OSC 111405, GenBank AY918956). **PARATYPES: UNITED STATES. California: El Dorado Co.** Above Hwy 49 S of American River Crossing, Trappe 3907, 18 May 1974 (OSC). **Fresno Co.** Sierra National Forest, Ross Creek drainage, Turtle Cr., K. Pendleton, L. Baker, B. Oakley, 25 June 1997, Trappe 22599 (OSC). **Sonoma Co.** Kelley Road, S. of Asti, R. Stone, 5 Nov 1976, Trappe 4912 (OSC). **Oregon: Benton Co.** Oak Creek, C. Wheeler, 18 Apr 1978, Trappe 5195 (OSC). **Douglas Co.** North Umpqua River, Oak Flat, L. & I. Spaulding 5 May 1984, Trappe 7881 (OSC). **Jackson Co.** Medford, Whetstone Savanna Preserve (42°25'N, 122°54'W) north of Medford, J. L. Frank 6 April 2004, 662 (SOC, OSC 111403); 15 April 2004, 682; 27 April 2004, 702 (SOC); 30 April 2004, 733 (SOC, OSC 111404); 31 May 2004, 747 (SOC, OSC 111406). **Linn Co.** Peterson's Butte, D. Wheeler, 21 June 1987, Trappe 9572 (OSC). **Marion Co.** Turner, Marion Hill Road, O. Bynum, 28 June 1980, Trappe 5854 (OSC). **Polk Co.** Rickreall Creek, W. Bushnell, 20 June 1987, Trappe 9559 (OSC). **Yamhill Co.** Champoeg State Park, A. Beyerle, 17 June 2000, Trappe 27170 (OSC 80215).

COMMENTS – *Tuber quercicola* is closely related to *T. candidum* and to the European spiny-spored species, *T. rufum* Pico and *T. ferrugineum* Vittad. *T. quercicola* with a rough, scaly, red peridium, and *T. candidum* with a smooth, tan peridium can fruit together under the same tree. The ITS region of *T. quercicola* is distinct from that of *T. candidum* with less than 90% of base pairs in common in the ITS1 and ITS2 regions. Molecular data position *T. quercicola* with *T. candidum* in a different clade from *T. rufum* and *T. ferrugineum* (Figure 7).

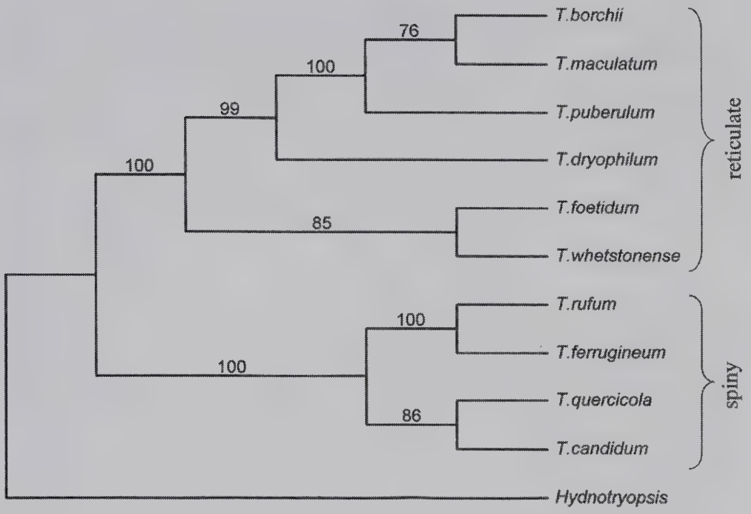


Figure 7. Phylogenetic tree of ten species in the genus *Tuber* constructed using maximum parsimony with bootstrap frequencies (values greater than 50%), comparing ITS sequences from three southern Oregon species (*T. candidum, T. quercicola* and *T. whetstonense*) to sequences of European species in GenBank, *T. borchii* (AF132505), *T. maculatum* (AF003909), *T. puberulum* (AJ557534), *T. dryophilum* (AF003917), *T. foetidum* (AJ557544), *T. rufum* (AF106892), *T. ferrugineum* (AF132506). Outgoup, *Hydnotryopsis setchellii* (AY927852).

Tuber whetstonense J.L. Frank, Southworth & Trappe **sp. nov.** **Figures 8-13**

Ascomata stereothecia hypogaea, globosa vel subglobosa, 5-15 mm lata, cartilaginea vel firma. Peridium minute furfuraceum, saepe rimosum, brunneolum vel obscure castaneum; suprapellis irregularis, cellulis versiformibus usque ad 4-8 (-10) μm latis; pellis 30-60 μm crassa, cellulis irregularibus vel isodiametris, 3-7 (-12) μm latis; subpellis 70-180 μm crassa, hyphis intertextis 2-4 (-6) μm latis. Gleba fuscum, venis albis marmorata. hyphis hyalinis intertextis 2-4 (-6) μm latis. Asci globosi, ellipsoides vel ovoides, 50-80 x 40-60 μm, sporis 1-5, maturitate parietes 1-2 μm crassis. Sporae aureobrunneae, ellipsoideae, in ascis unisporis 42-55 x 34-44 μm sine ornamentato, in ascis bisporis 30-40 x 26-38 μm, in ascis trisporis 24-34 x 20-30 μm, in ascis quadri- vel quinquesporis 15-26 x 14-22 μm; ornamentum reticulatum alveolatum 2-4 μm altum, alveolis 9-12 secus longitudinem sporarum. Holotypus J. L. Frank 756 (SOC).

Etymology: in reference to the type locality, Whetstone Savanna Preserve.

Ascomata globose to subglobose stereothecia 5-15 mm broad, rubbery to firm. **Peridium** minutely scurfy and pubescent with cracks and depressions, often cracked along the ascocarp perimeter, light brown to dark reddish brown. **Gleba** solid, with broad, dark grayish brown fertile tissue marbled with narrow white veins. **Odor** mildly peppery. **Taste** not recorded.

Peridium 180-240 μm thick: **suprapellis** at maturity an irregular surface of versiform cells 4-8 (-10) μm broad and with golden brown walls 1-2 μm thick; **pellis** 30-60 μm thick, a pseudoparenchyma of irregular to isodiametric cells 3-7 (-12) μm broad with golden brown walls 1-2 μm thick near the suprapellis grading to hyaline near the subpellis; **subpellis** intergrading from the pellis, 70-180 μm thick, of hyaline, thin-walled, interwoven hyphae 2-4 (-6) μm broad, in places arranged parallel to the ascomatal surface. **Glebal trama** of hyaline, thin-walled, interwoven hyphae 2-4 (-6) μm broad, the sterile veins of similar hyphae, mostly parallel to subparallel. **Asci** globose to ellipsoid or ovoid 50-80 x 40-60 μm, 1-5 spored, hyaline, lacking a stem or sometimes with a stem up to 20 x 10 μm, walls 1-2 μm thick at maturity, embedded randomly through the tramal tissue.

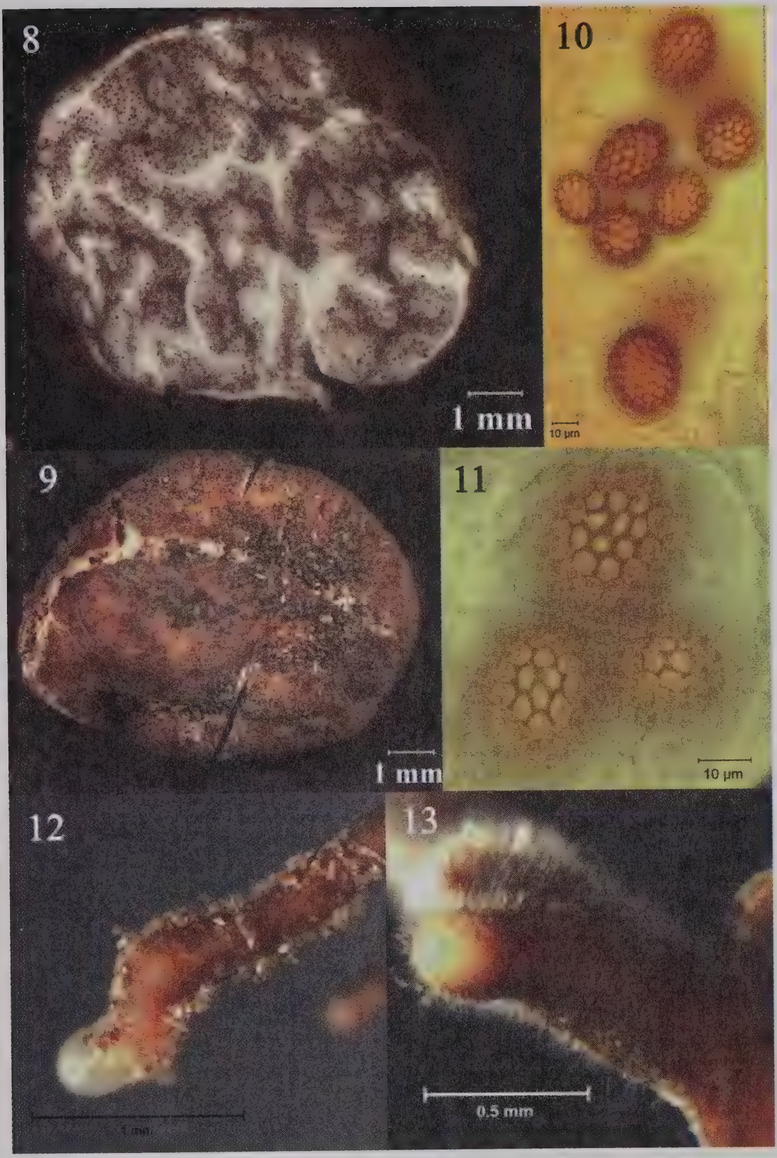
Spores golden brown, subglobose to ellipsoid, 15-55 x 14-44 μm excluding ornamentation and depending on number of spores per asci: in 1-spored asci 42-55 x 34-44 μm, 2-spored 30-40 x 26-38 μm, 3-spored 24-34 x 20-30 μm, and 4-5-spored 15-26 x 14-22 μm; **ornamentation** an alveolate reticulum 2-4 μm tall with 9-12 meshes along the spore length; spore walls 3-5 μm thick.

Ectomycorrhizal morphotype tan to brown with lighter tips, the surface with septate cystidia 50-60 μm long. **Outer mantle** an interlocking irregular synenchyma to net synenchyma. **Inner mantle** a non-interlocking irregular synenchyma to regular synenchyma.

> **Collections examined: HOLOTYPE — UNITED STATES. OREGON: Jackson Co.**, MEDFORD, Whetstone Savanna Preserve (42°25'N, 122°54'W) 1 June, 2004. J. L. Frank 756 (SOC, isotype, OSC 111412, GenBank AY830855). **PARATYPES: UNITED STATES. OREGON: Jackson Co.**, MEDFORD Whetstone Savanna Preserve (42°25'N, 122°54'W). J. L. Frank, 3 June 2004, 762 (SOC, OSC 111413), D. Southworth, 17 May 2000, 40; 13 April 2001, 148 (SOC).

COMMENTS – The distinctive morphological characters of *T. whetstonense* are its fine-meshed spore reticulum (9-12 meshes along the spore length) in combination with a brown, pseudoparenchymatic peridiopellis, relatively thick-walled asci, and association with *Quercus garryana* in xeric habitats. It resembles *Tuber anniae* Colgan & Trappe, but the latter species has smaller, globose to subglobose spores, a glabrous, yellow to dark olive brown peridium, and a peridiopellis with scattered cells inflated up to 20 μm broad. Moreover, *T. anniae* inhabits mesic conifer forests in contrast to *T. whetstonense,* an inhabitant of xeric oak communities. *Tuber pacificum* Trappe et al. also has spores with a fine-meshed reticulum and thick-walled asci, but its peridium has a suprapellis of appressed to tangled hyphae, and it occurs in mesic conifer forests. A number of other small and not very distinctive *Tuber* collections at OSC are similar to the species noted above but differ in various respects. Evidently, these small species comprise a complex as morphologically vexing as that found for similar species in Europe (Halász et al. 2005).

Of the *Tuber* species in Oregon oak woodlands, *T. whetstonense* is morphologically and molecularly distinct from the more common, spiny-spored species *T. candidum* and *T. quercicola*. The length of its ITS region is shorter than 600 bp, whereas those of *T. candidum* and *T. quercicola* are longer than 700 bp. Molecular analysis with ITS data


8
10
1 mm
10 µm
9
11
1 mm
10 µm
12
13
1 mm
0.5 mm

Figures 8–13. *Tuber whetstonense* sp. nov. **Figure 8.** Cross-section (SOC756). **Figure 9.** External surface. **Figures 10–11.** Ascospores stained with Melzer's reagent. (SOC756). **Figure 12.** Mycorrhiza (MAT0502) showing light tip. **Figure 13.** Mycorrhiza (MAT0502) showing cystidia.

separates the spiny-spored species from the reticulate-spored species, indicating two major clades within the genus *Tuber* (Figure 7). *T. whetstonense* fruited rarely: in five years, 4 sporocarps were collected, compared with over 200 for *T. candidum* and 32 for *T. quercicola*.

The mycorrhizal status of *Tuber whetstonense* was confirmed by DNA sequences. ITS sequence data from the holotype (J. L. Frank 756 [SOC]; OSC 111412; GenBank AY830855) matched a mycorrhiza (MAT0502; GenBank AY969006) collected from *Quercus garryana* roots at Whetstone Savanna Preserve; the two sequences are greater than 98% similar.

Ecology

Hypogeous fungi are common in Mediterranean climates, having evolved under selection pressures for survival in warm, dry climates (Montecchi & Sarasini 2000, Trappe & Claridge 2005). Subterranean fruiting strategies to conserve moisture require alternatives to the aerial spore dispersal mechanism common to epigeous mushrooms. Animals, attracted by volatile aromatics produced by the hypogeous fruiting bodies at maturity, dig them up, consume them, and disperse the spores in their feces (Claridge & Trappe 2005).

Analysis of fecal pellets of three small mammals, *Microtus californicus*, *Peromyscus maniculatus,* and *Reithrodontomys megalotis,* revealed that the distinctive, large, spiny spores of *T. candidum* were among the more frequently observed fungal spores in fecal pellets. Spores from *T. quercicola* and *T. whetstonense* were also present in pellets (Frank et al. 2004; Frank 2005).

Acknowledgements

Seth Barry and Lori Valentine provided mycorrhizal data from Whetstone Savanna Preserve. David Oline and Kathleen Page, Southerrn Oregon University, and Peter Kennedy, University of California, Berkeley, assisted with molecular analysis. Matthew E. Smith, University of California, Davis, assisted with molecular analysis and shared data from collections at the Sierra Nevada Research site. Karen Hansen, Harvard University Herbaria, made useful suggestions about the manuscript, in particular regarding molecular methods and phylogenetic analysis. Greg Bonito, Duke University, discovered in his sequences of North American *Tuber* species that a collection identified as *Tuber anniae* had a sequence identical to that of *T. whetstonense.* We rechecked the specimen and found it to be, in fact, *T. whetstonense.* He also made helpful suggestions about the manuscript.

We thank The Nature Conservancy for access to the study site. This material is based upon work supported by the National Science Foundation under Grants No. 9981337, DEB-0516229 and DBI-0115892, and by NATS, the North American Truffling Society (to JLF). Dr. Trappe's participation in the research was supported in part by the USDA Forest Service Pacific Northwest Research Station, Forestry Sciences Laboratory, Corvallis, Oregon.

Literature Cited

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. 1990. Basic local alignment search tool. J Mol Biol 215: 403-410.

Brundrett M, Bougher N, Dell B, Grove T, Malajczuk N. 1996. Working with mycorrhizas in forestry and agriculture. ACIAR Monograph 32. Pirie Printers. Canberra, Australia.

Castellano MA, Trappe JM, Maser Z, Maser C. 1989. Key to spores of the genera of hypogeous fungi of north temperate forests with special reference to animal mycophagy. Mad River Press, Eureka, California.

Ceruti A. 1961. Revisione di alcune specie di *Elafomicetale* e di *Tuberali* dell'America del Nord. Allionia 7: 1-25.

Ceruti A, Fontana A, Nosenzo C. 2003. Le specie europee del genere *Tuber*–Una revisione storica. Museo Regionale di Scienze Naturali, Torino.

Claridge AW, Trappe JM. 2005. Sporocarp mycophagy: nutritional, behavioral, evolutionary and physiological aspects. In J Dighton, JF White & P Oudemans (eds), The Fungal Community—Its Organization and Role in the Ecosystem, Taylor & Francis, Boca Raton, Florida. pp. 599-611.

Frank J, Petersen C, Berninghausen H, Southworth D. 2004. Oaks, hypogeous fungi and mammal mycophagy: complex mutualism. Abstract, 89th Annual Meeting of the Ecology Society of America. Portland, Oregon.

Frank JL. 2005. Complex mutualism in an Oregon white oak woodland: hypogeous fungi, mycorrhizas and small mammal mycophagy. Masters Thesis, Southern Oregon University, Ashland, Oregon.

Gardes M, Bruns TD. 1993. ITS primers with enhanced specificity for basidiomycetes- application to the identification of mycorrhizae and rusts. Mol Ecol 2: 113-118.

Goodman DM, Durall DM, Trofymow JA, Berch SM. 1996. A manual of concise descriptions of North American ectomycorrhizae. Mycologue Publications Sydney, Australia.

Halász KZ, Bratek D, Szeg S, Rudnóy I, Rácz D, Lásztity, Trappe JM. 2005. Tests of species concepts of the small, white, European group of *Tuber* species based on morphology and rDNA sequences with special reference to *Tuber rapaeodorum*. Mycological Progress 4: 291-298.

Harkness HW. 1899. California hypogeous fungi. Proc. Calif. Acad. Sci. Ser. 3 Bot. 1: 241-292.

Lange M. 1956. Danish hypogeous macromycetes. Dansk Bot Ark 16: 1-84.

McCarthy C. 1998. Chromas 1.45. School of Health Science, Griffith University, Southport, Queensland, Australia.

Montecchi A, Sarasini M. 2000. Funghi ipogei d'Europa. Fondazione Centro Studi Micologie dell'A. M.B., Vicenza, Italy.

Swofford, DL. 2001. PAUP*: Phylogenetic analysis using parsimony and other methods, 4.0 10 ed. Sinauer Associates, Sunderland, Massachusetts.

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG. 1997. The Clustal X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res 24: 4876-4882.

Trappe JM. 1968. Relazioni fra le specie di tartufi europee e nord-americane. Ati Congresso Internazionale sul Tartufo, Spoleto. pp. 134-137.

Trappe JM, Claridge AW. 2005. Hypogeous fungi: evolution of reproductive and dispersal strategies through interactions with animals and mycorrhizal plants. In J Dighton, JF White & P Oudemans (eds), The Fungal Community—Its Organization and Role in the Ecosystem. Taylor & Francis, Boca Raton, Florida. pp. 613-623.

Valentine LL, Fiedler TL, Hart AN, Peterson CA, Berninghausen HK, Southworth D. 2004. Diversity of ectomycorrhizas associated with *Quercus garryana* in southern Oregon. Can J Bot 82: 123-135.

Weber NS, Trappe JM, Denison WC. 1997. Studies on western American *Pezizales*. Collecting and describing ascomata—macroscopic features. Mycotaxon 61: 153-176.

White TJ, Bruns T, Lee S, Taylor J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: MA Innis, DH Gelfand, JJ Sninsky, TJ White (eds). PCR Protocols: a guide to methods and applications, pp. 315-322. Academic Press, New York.

Zobel JG. 1854. Augusti Car. Jos. Corda, Iconum Fungorum Hucusque Cogitorum Tomus VI. (Ultimus). Libraria Friderici Ehrlich, Prague.

MYCOTAXON

Volume 95, pp. 241–245 January–March 2006

Two new species of *Parmotrema* (*Parmeliaceae*, Lichenized Ascomycota) from Brazil

Cristine G. Donha & Sionara Eliasaro

sionara@ufpr.br
Depto. Botânica, Biológicas, Universidade Federal do Paraná
Cx. P. 19031, 81531-970, Curitiba, PR – Brazil

Abstract—*Parmotrema sorediosulphuratum* and *Parmotrema superaguiense* are described as new. Both species were collected in Guaraqueçaba Environmental Protection Area, located in the northern coast of the Paraná State, southern Brazil. The former species was found in areas of the atlantic rain forest at 40 m altitude and the latter one was found only in the Parque Nacional do Superagüi in restinga, a characteristic type of vegetation occurring on nutrient-poor sandy soils along the Brazilian coastline. The species are morphologically and chemically characterized and illustrated. Comments with related species are also presented.

Key words—eumitrin, lichens, pigments

Introduction

Parmotrema A. Massal. is one of the most prominent group of foliose lichens in Brazil, and is mainly characterized by the large thallus with broad lobes, broad naked marginal zone on the lower surface, simple to rarely branched rhizines and sublageniform to filiform conidia (Elix 1993). The medulla in the majority of the species is totally white or sometimes with an orange to orange-red coloration mainly near the lower cortex, due to the presence of anthraquinones and related substances that react K+ purple. However, there are 34 species with a totally or partially yellow or yellow-orange pigmented medulla that contain pigments such as pulvinic acid and its derivatives and the ergochromes that do not react with K (Hale 1965, 1974, 1977, 1986, Kurokawa 1974, 1984, Krog & Swinscow 1981, 1983, Louwhoff & Elix 1999, 2002, Nash III & Elix 2002). These species of *Parmotrema* with a yellow medulla form a heterogeneous group that belongs to several different phylogenetic lines (Hale 1974).

During an extensive study of the taxonomy and distribution of the family *Parmeliaceae* in Paraná State, two new species of *Parmotrema* with yellow pigments in the medulla were discovered. These species are described as new based upon their morphological and chemical characteristics.

Materials and Methods

The two new species were described from specimens collected in Guaraqueçaba Environmental Protection Area (24°45'–25°30'S and 48°00'–48°45'W), located on the northern coast of the Paraná State, southern Brazil. This region represents the best-preserved area of continuous Atlantic forest left in Brazil. The different types of habitats in the area include mangroves, restinga (a characteristic type of vegetation occurring on nutrient-poor sandy soils along the Brazilian coastline), lowland forest, submontane, montane, and upper montane forests. The climate of the region is humid subtropical (IPARDES 1990).

Collections of the new species were deposited in UPCB. Specimens of related species borrowed from S were examined for comparison. The specimens were examined with a dissecting microscope for morphological characterization. Apothecia and pycnidia were cut by hand with a razor blade and observed under a light microscope to examine the anatomical characters. Lichen substances were identified by thin layer chromatography according to the methods standardized for lichen products (Culberson & Ammann 1979, Elix & Ernst-Russell 1993) and comparison with authentic samples.

Taxonomic Description

Parmotrema servediosulphuratum Eliasaro & Donha **sp. nov.** **Fig. 1**

> *Thallus membranaceus ad subcoriaceum, adnatus, usque 14 cm lati. Lobi subirregulares ad sublineares, marginibus crenatis ad fortitutos sublaciniatus, moderatis ad densos ciliatis; cilia maxime simplicia. Superficies supra viridis cineracea, immaculata. Soralia marginalia et submarginalia. Medulla flava sulphurea. Superficies infra nigra cum marginibus brunneis atris ad claros; rhizinae simplices ad furcatas. Apothecia et pycnidia ignota. Atranorinam, acidum vulpinicum et pigmenta flava ignota continens.*
>
> **Type: BRAZIL. Paraná: Antonina.** Chácara donha (25°14'31"S 48°44'49"W) – 29.IX.2004, *C G Donha 1808* (**HOLOTYPE**-UPCB).

Thallus corticolous, membranaceous to subcoriaceous, adnate, up to 14 cm wide. Lobes subirregular to somewhat sublinear, plane or ascending, 4-10 mm wide, with rotund apices, margins crenate to sublaciniate, moderate to densely ciliate; cilia black, mainly simple or bifurcate, rarely trifurcate, 1.0-3.5 mm long. Upper surface grey green, emaculate, smooth, becoming rugose and irregularly cracked towards the center. Soralia marginal and submarginal, soredia granular to subgranular. Medulla bright sulphur-yellow. Lower surface black with a dark to pale brown margins, smooth to rugose, moderately rhizinate with a narrow bare marginal zone, 1.0-4.5 mm wide; rhizines black, in scattered groups, simple to furcate, 0.5-1.0 (-2.0) mm long. Apothecia and pycnidia not seen.

Chemistry: cortex: K+ yellow (atranorin); medulla: K-, C-, KC-, UV- (vulpinic acid, two undetermined yellow pigments).

Ecology, Range and Distribution – *Parmotrema sorediosulphuratum* is known only from the type locality, where it grows on tree trunks in areas of atlantic rainforest at 40 m altitude.

> **REPRESENTATIVE SPECIMENS EXAMINED** – **BRAZIL. Paraná: Antonina.** Chácara donha (25°14'31"S 48°44'49"W) – 03.VII.2003, *C G Donha 972* (UPCB). Specimen examined for comparison: **BRAZIL. Mato Grosso: Jangada.** Fazenda Santa Elina – VII.2000, *G Ceccantini s.n.* (*Parmotrema cornutum* (Lynge) Hale-UPCB).

Comments – *Parmotrema sorediosulphuratum* is characterized by the ciliate lobes, the marginal and submarginal soralia and by the intensely sulphur yellow medulla due to the production of vulpinic acid. The occurrence of vulpinic acid in *Parmotrema* species is rare and known only in two another species, *P. cornutum* (Lynge) Hale and *P. sulphuratum* (Nees & Flot) Hale. *Parmotrema sorediosulphuratum* differs from both by the production of soredia and may be considered the sorediate counterpart of *P. cornutum.*

Parmotrema superaguiense Donha & Eliasaro **sp. nov.** **Fig. 2**

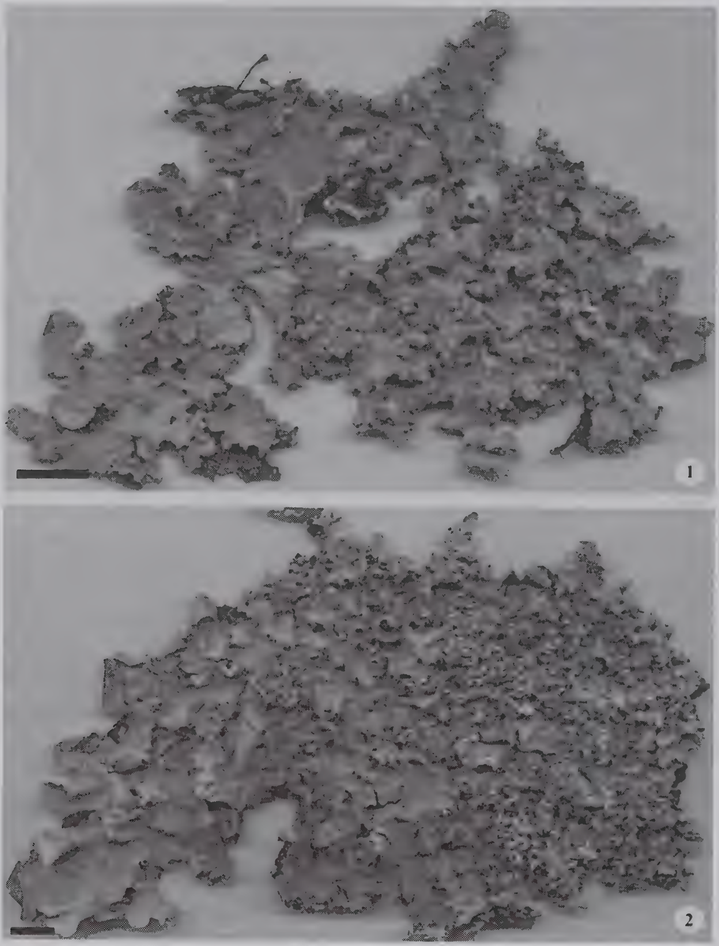
> *Thallus membranaceus, laxe adnatus, usque 16 cm lati. Lobi irregulares 10-20 mm lati, marginibus moderatis ciliatis; cilia maxime simplicia. Lacinulae frequentes, maxime in centrum thalli, simplices ad irregulares ramosas. Superficies supra viridis cineracea, immaculata. Isidia et soredia absentes. Medulla flava. Superficies infra nigra cum marginem brunneum clarum ad brunneum flavum; rhizinae simplices. Apothecia in laminatiam, pedicellata ad subpedicellata, discus imperforatus, margo thallinus eciliatus fortuitus laciniatus, spori 20,0-27,5 (-32,5) x 10,0-15,0 µm. Pycnidia frequentia, conidia sublageniformia 6-8 x 1 µm. Atranorinam, acidum graxum et duo pigmenta flava continens.*
>
> Type: **BRAZIL. Paraná: Guaraqueçaba.** Parque Nacional do superagüi, Ilha das Peças (25°28'20"S 48°17'58"W) – 24.IV.2004, *S Eliasaro 2755* (**HOLOTYPE**-UPCB).

Thallus corticolous, membranaceous to subcoriaceous, loosely adnate, 12-16 cm broad. Lobes irregular, plane to ascending, 10-20 mm wide, with rotund apices, margins entire to laciniate toward the center of the thallus, laciniae 0.5-1.5 mm wide, moderately ciliate; cilia black, mainly simple, rarely furcate, robust, 0.5-2.0 mm long, mainly in lobes axils. Upper surface greenish gray, entire, smooth to rugose on older lobes. Isidia and soredia lacking. Medulla deep yellow. Lower surface black with a yellowish brown margin, smooth to rugose, sparsely rhizinate with a broad, bare marginal zone, up to 10 mm wide; rhizines black, in scattered groups, simple, up to 1 mm long. Apothecia common, laminal, stiptate to substiptate, disc imperforate, 2-10 mm in diameter, thalline margin eciliate, entire to rarely laciniate, laciniae often pycnidiate; amphithecium maculate, sometimes rugose veined; spores 20.0-27.5 (-32.5) x 10.0-15.0 µm. Pycnidia common, submarginal on lobes and lacinulae; conidia sublageniform, 6-8 x 1 µm.

Chemistry: cortex K+ yellow (atranorin); medulla K-, C-, KC-, UV- (fatty acid, eumitrin B and eumitrin A group of pigments).

Ecology, Range and Distribution – *Parmotrema superaguiense* is so far only known from the Parque Nacional de Superagüi, in restinga.

> **REPRESENTATIVE SPECIMENS EXAMINED** – **BRAZIL. Paraná: Guaraqueçaba.** Parque Nacional do superagüi, Ilha das Peças (25°28'20"S 48°17'58"W) –24.IV.2004, *S Eliasaro 2748* (UPCB); Ilha de Superagüi (25°27'S 48°14'W) – 8.IV.2003, *S Eliasaro & C G Donha 2589* (UPCB); 10.IV.2003, *S Eliasaro & C G Donha 2706* (UPCB); 15.VIII.2004, *C G Donha 1832* (UPCB). Specimens examined for comparison: **BRAZIL. Mato Grosso: Coxipó,** near Cuiabá – *Malme 2198B* (holotype-S, *Parmelia lyngeana* Zahlbr.: based on *Parmelia merrillii* Lynge, 1914, *non Parmelia merrillii* Vain., 1909); **Santa Anna da Chapada** – *Malme 2477* (holotype-S, *Parmelia cornuta var. crocea* Lynge).



Figures 1-2. New species of *Parmotrema*: 1. *Parmotrema sorediosulphuratum* (holotype in UPCB). 2. *Parmotrema superaguiense* (holotype in UPCB). Bars = 10 mm.

Comments – *Parmotrema superaguiense* is characterized by the yellow medulla, the ciliate lobe margins, the presence of laciniae and the imperforate apothecial disc. It is superficially similar to *P. lyngeanum* (Zahlbr.) Hale, which has narrow lobes (5-10 mm wide) and the holotype produces protocetraric acid and skyrin. Other ciliate *Parmotrema* species that lack vegetative propagules and have a distinctly yellow to orange yellow medulla differ from *P. superaguiense* in chemical properties and other characters: *P. lopezii* Hale has a yellowish green thallus with usnic acid and atranorin in the upper cortex and protocetraric acid and secalonic acid A in medulla. *P. cristatum* (Nyl.) Hale contains protocetraric acid in medulla. *P. appendiculatum* (Fée) Hale produces barbatic acid in the medulla and, according to Krog & Swinscow (1983), a pigment restricted to the vicinity of the apothecia and the thalline exciple.

Acknowledgements

The authors wish to thank the curator of S herbarium for the loan of specimens, Prof. Nasser K. Hammad for checking the Latin descriptions; Dr. John A Elix and Dr. Susana Calvelo for the critical revision of the manuscript and "Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis" (IBAMA) for collection permission nº 118/2002 and 133/2003. Cristine G. Donha is grateful to "Coordenação de Aperfeiçoamento de Pessoal de Nível Superior" (CAPES) for a master fellowship.

Literature Cited

Culberson CF, Ammann K. 1979. Standarmethode zur Dünnschicht-chromatographie von Flechtensubstanzen. Herzogia 5: 1-24.

Elix JA. 1993. Progress in the generic delimitation of *Parmelia sensu lato* lichens (Ascomycotina: Parmeliaceae) and a synoptic key to the *Parmeliaceae*. Bryologist 96: 359-383.

Elix JA, Ernst-Russell KD. 1993. A catalogue of standardized thin layer chromatographic data and biosynthetic relationships for lichen substances. Australian National University, Canberra.

Hale ME. 1965. A monograph of the *Parmelia* subgenus *Amphigymnia*. Contributions from the United States National Herbarium 36: 193-358.

Hale ME. 1974. Notes on species of *Parmotrema* (Lichenes: Parmeliaceae) containing yellow pigments. Mycotaxon 1: 105-116.

Hale ME. 1977. New species in the lichen genus *Parmotrema* Mass. Mycotaxon 5: 432-448.

Hale ME. 1986. New species in the lichen family *Parmeliaceae* (Ascomycotina). Mycotaxon 25: 85-93.

[IPARDES] Instituto Paranaense de Desenvolvimento Econômico e Social. 1990. Macrozoneamento da Área de Proteção Ambiental de Guaraqueçaba. Curitiba.

Krog H, Swinscow TDV. 1981. *Parmelia* subgenus *Amphigymnia* (Lichenes) in East Africa. Bulletin of British Museum of Natural History (Bot.) 9: 143-231.

Krog H, Swinscow TDV. 1983. A new species and new combinations in *Parmotrema* (*Parmeliacae*). Lichenologist 15: 127-130.

Kurokawa S. 1974. Four new species of *Parmelia* from Brazil. Bulletin of the National Science Museum (Tokyo) 17: 297-300.

Kurokawa S. 1984. Joint occurrence of diffractaic and barbatic acids in *Parmelia*, subgenus *Amphigymnia* (Lichenes). Journal of Japanese Botany 59: 193-198.

Louwhoff SHJJ, Elix JA. 1999. *Parmotrema* and allied lichen genera in Papua New Guinea. Bibliotheca Lichenologica 73.

Louwhoff SHJJ, Elix JA. 2002. The *Parmeliaceae* (lichenized Ascomycota) of New Caledonia. Lichenologist 34: 373-394.

Nash III TH, Elix JA. 2002. *Parmotrema*. In: Lichen Flora of the Greater Sonoran Desert Region (TH Nash III, BD Ryan, C Gries, F Bungartz, eds.). ASU Lichen Herbarium, Tempe, v.1, p.318-329.

MYCOTAXON

Volume 95, pp. 247–254 January–March 2006

Russula siamensis: a new species of annulate *Russula* from Thailand

S. Yomyart[1], J. Piapukiew[2], R. Watling[3],
A. J. S. Whalley[4] & P. Sihanonth[5]✧

✧*sprakits@chula.ac.th*
[1]*Program of Biotechnology, Faculty of Science, Chulalongkorn University*
Bangkok 10330, Thailand

[2]*Department of Botany, Faculty of Science, Chulalongkorn University*
Bangkok 10330, Thailand

[3]*Caledonian Mycological Enterprises, Crelah*
26 Blinkbonny Ave, Edinburgh EH4 3HU. Scotland, U.K.

[4]*School of Biomolecular Science, Liverpool John Moores University*
Byrom Street, Liverpool, L3 3AF, U.K.

[5]*Department of Microbiology, Faculty of Science, Chulalongkorn University*
Bangkok 10330, Thailand

Abstract —*Russula siamensis*, associated with *Dipterocarpus alatus*, is described as new. An annulate *Russula* is unique in the Thai mycota.

Key words—*PellicularIae, Ingratae, Pectinatae*

Introduction

Annulate species of *Russula* Pers. are totally unfamiliar to European field mycologists except for a privileged few who can collect in the tropics, yet Europe was the continent that spawned the basic classification of the genus. Studies there attempted to organize, in what was thought to be a natural way, those species found in the temperate areas of the world; for a recent account see Romagnesi (1967). As exploration was made more distant from Europe collections were made which stretched this classification to breaking point. No more so when Heim (1938ab) described material from Madagascar when it soon became clear that all was not well with the previously held, rather simplified, views. Heim described a range of species of *Russula* which possessed rings and which he clustered around his names '*Russula annulata, R. heliochroma & R. parasitica*'. Even then he adopted a rather cautious approach in dealing with these fungi, although Heim was often prepared to accept rather unorthodox and controversial views (Heim 1948, 1971). Species with an annulus were even a novelty to him and he created the new Sect. *Pelliculariae* to house the species he studied emphasising the thin-flesh, pectinate pileus-margin and an annulus, which could be very fugitive indeed.

The next stage in the development of this saga was the description from South America of *Russula brasiliensis* Singer, a fungus later found by to be the same as *Clitocybe puiggarii* Speg. and subsequently the epithet was transferred to *Russula* by Singer, similarities in structure lying with other *Pellicularaiae*; see Singer (1986). There is little doubt that Mme. Goossens-Fontana when resident in the then Belgian Congo had been familiar with annulate russules and had illustrated them, the description of *R. xylophila* Beeli and its allies resulting from these observations (Beeli 1928, 1936). It was not until one of us (RW) visited the Cameroon, West Africa, and Buyck published on the East African and Congo *Russula* mycota (Buyck 1993, 1994) that the number of annulate *Russula* species became awe-inspiring. Thirteen annulate species have now been formally described from Africa and several more are known from field notes. Subsequently *Russula puiggarii* was collected in the Caribbean by Pegler (1983), along with a second new species, *R. hygrophytica* Pegler (Pegler & Singer 1980). Buyck & Ovrebo (2002) in their study of Russula species from Panama considered *R. puiggarii* and *R. hygrophytica* to be conspecific but Pegler (pers. comm.) maintains that they are distinct taxa. A whole raft of S. American taxa all attributed to Sect. *Pellicularaiae*, some with rings and others closely related lacking such a structures, are now known.

In the *Pellicularaiae* Singer (1986) recognized eight subsections, viz. *Epitheliosae* Singer, *Guayarenses* Singer, *Radicantes* R. Heim, *Heliochrominae* R. Heim, *Discopodinae* R. Heim, *Diversicolores* Singer, *Pluviales* Singer and *Batistae* Singer. Subsections *Heliochrominae* and *Discopodinae* and possibly *Pluviales* contain representatives from both Africa and from South America, whilst the others are based on S. American elements—except subsect. *Guayarenses*, which includes a single species from Australasia, viz. *R. eburneoareolata* Hongo. Thus this last taxon was the only Asiatic species then known in the group. Since Singer's summary, Buyck & Horak (1999) have described *R. cingulata* from Papua New Guinea, and although it would have been placed within Heim's concept of Sect. *Pellicularaiae*, these authors placed their new taxon in Sect. *Fistulosae* subsect. *Meleagrinae* in keeping with the re-evaluation of pellicularioid *Russula* spp. The species are distributed, as Buyck has eloquently demonstrated, not just in one section, viz. *Pellicularaiae*, but in several sections of *Russula* indicating that the annulate/thin-fleshed morphotype had evolved several times, probably in response to environmental factors (Buyck 1988ab).

The present article reports for the first time on an annulate *Russula* from Thailand indicating that the annulate feature is a more widely distributed phenomenon than first thought, exposing still further the inadequacies of our present approach to understanding the genus *Russula* based on European taxa alone. The finding of such a fungus was in the opinion of one of us inevitable, as elements had already been found amongst the Malaysian *Russula* mycota that demonstrated copious veil production attached to the pileus-surface in much the same way as the recently described *R. messapica* Sarnari from Italy.

Materials and Methods

During collecting trips to plantations of *Dipterocarpus alatus* Roxb. (*Dipterocarpaceae*) in eastern Thailand, several interesting mycorrhizal fungi have been collected. One of these was a species of *Russula* characterised by a distinctly annulate stipe. Its basic appearance was one of a thin-capped pileus with pectinate-striate margin, slender stature and isolated spines on the basidiospores. These fundamental characters agree in all ways with the Neotropical members of the Sect. *Pelliculariae,* but further analysis was necessary for placement in the scheme proposed by Buyck et al. (1986). The present species grows on bare sandy loam soil with *D. alatus* which prefers moist soils, and with ground cover of Malaysian grass.

DNA analysis

Genomic DNA was prepared from the fresh stipe tissue and extracted with cetyltrimethylammonium bromide (CTAB) as described in Zhou et al. (1999). PCR amplification of the internal transcribed spacer (ITS) was performed in a total volume of 50 μl which comprised approx. 100 ng genomic DNA, 1× PCR Master Mix (Fermentas, California, USA), and the primer ITS1f (Gardes & Bruns 1993) and ITS4 (White et al. 1990). The amplification was performed in a thermocycler (TGradient; Biometra, Germany) with 94 °C for 5 min , followed by 38 cycles of 94 C for 1 min, 51 C for 1 min and 72 C for 1 min, with a final extension of 72 C for 5 min. PCR product was purified using the NucleoSpin® (Macherey-Nagel Inc., Easton, USA) and sequenced externally by Macrogen (Seoul, Korea) using the same primers as for amplification. The ITS sequence of *R. siamensis* was submitted to GenBank with accession number AB206535.

Taxonomic Description

Russula siamensis Yomyart, Piapukiew, Watling, Whalley & Sihan. **sp. nov.**

Figs. 1-2

> *Pileus 28 mm, convexus dein depresso-explanatus, centro glabro sed conspicue sulcato marginum versus griseo-brunneus vel pallide fuscus, subviscidus. Stipes 18 x 7mm, cylindricus, lamellis concoloris, siccus, fragile, annulo distincto membranaceo mobili concolore instructis vel pileis-concolores. Lamellae adnatae - adnexae, subdensae, primo albidae dein pallide. Caro albida, tenua. Sapore leviter acri; Odore leviter frugose. Sporae albidae in cumulo 8 -9 x 6 -7 μm subglobosae vel ovoideae verruculosis cylindricis isolatis amyloideis suprahilaris inamyloidea. Basidia 4-sporigera. Cystidia cylindrica vel fusoideo-mucronata numerosa. Holotypus: Thailand, Khao Hin Sorn, Chacherngsoa prov. 22 viii 2004, sub Dipterocarpus alatus. Wat.No. 28784 in E.*

Pileus 28mm, convex then plano-convex but slightly and shallowly depressed at centre, not involute, pectinate-striate at margin, strongly striate to 2/3rds, brownish biege to pale ochraceous grey, darker along the radial ridges and then even hazel colour, slightly viscid when fresh, radially fibrillose-wrinkled but not disrupting to form flakes or squamules. Fig 2A; **stipe** 18 x 7mm, whitish, flushed with colour of pileus in smudges below annulus, cylindric, equal, with obtuse abrupt rounded base slightly depressed at point of attachment to the substratum, darkening at very base; **annulus** about 1mm thick with a lower zone concolorous with pileus and an upper white zone, membranous, loosening, discrete; **lamellae** adnate to adnexed, concolorous with stipe, cream-colour, relatively widely spaced, very few intermediates but some major lamellae venose at their base. Fig 2B; **context** very thin; **taste** slightly acrid; **odour** slightly fruity.

Basidiospores heterotropic orthotropic, white to very pale cream-colour in mass (A - B), 8 - 9 x 6 - 7 μm excl. ornamentation, subglobose to broadly ellipsoid with a slight suprahilar applanation, but lacking distinct plage, hyaline, with strongly amyloid ornamentation consisting of isolated or almost isolated conical warts 0.5-1µm high, neither catenulate nor reticulate, hilar appendage distinct < 2µm long with a basal slightly amyloid cuff. Figs. 1F & 2D; b/w photos; **cheilocystidia** crowded forming an almost completely homogeneous margin, 29 - 48 x (5.6-) 6.7 - 11 µm, hyaline, elongate-clavate, some drawn out into broad lanceolate apex or clavate with minute apical papilla, changing to either yellowish sepia in SV or where more deeply seated dark purple-brown. Figs. 1B, C & 2C; **macrocystidia** widespread, hyaline with or without granular contents in ammoniacal solutions, similar to cheilocystidia but more distinct, more elongate-clavate and appendages at apex more elaborate, often drawn out into

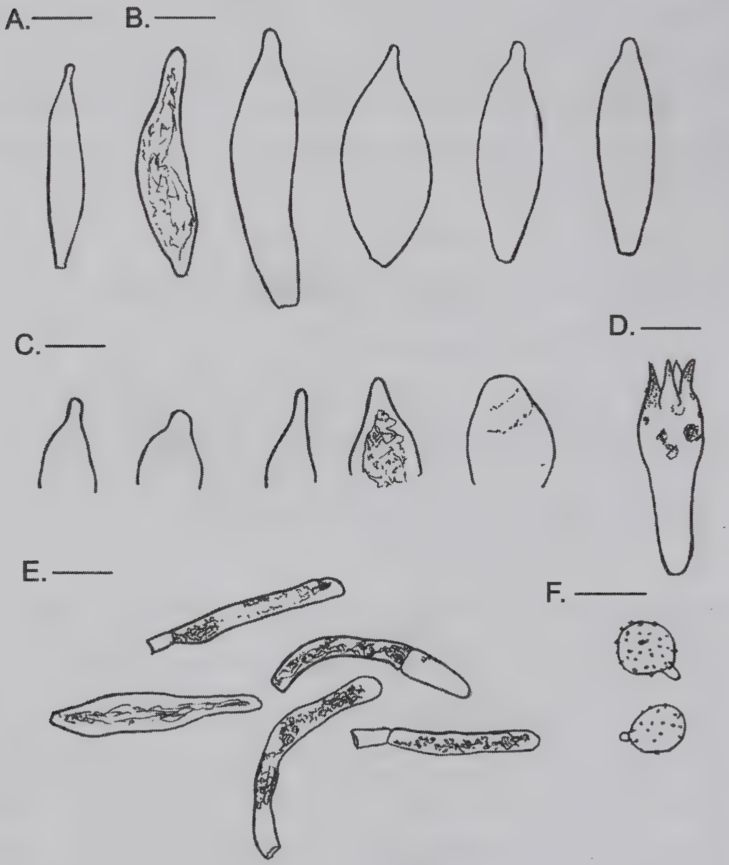


Fig. 1. *R. siamensis* holotype. A. Macrocystidium. B. Cheilocystidia. C. Range of apices of cheilocystidia. D. Basidium. E. Terminal elements of pileipellis. F. Basidiospores.

Bars: A-E = 10 µm; F = 5 µm.

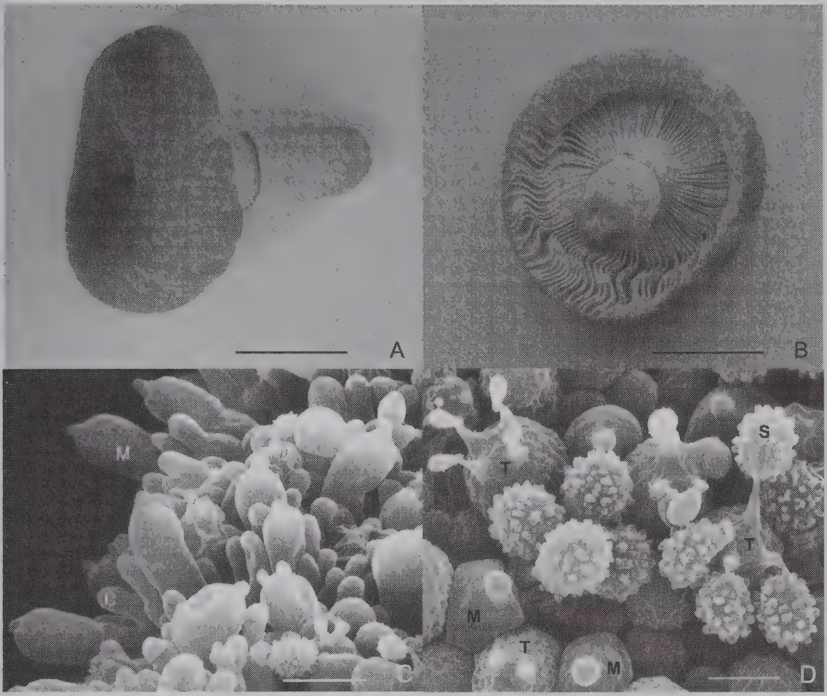


Fig. 2. *R. siamensis* holotype. A.&B. Fresh basidiome. C. Scanning electron micrograph of cheilocystidia (C), macrocystidia (M) of *R. siamensis*. D. High magnification of macrocystidia (M), basidiospore (S) and tetrasterigmatic basidia (T) with various stages of basidiospore formation of *R. siamensis*. Note: smooth surface of papillate macrocystidia compared to wrinkled surface of basidia. Bars: A = 10 mm; B&C = 10 µm; D = 5 µm.

elongate papilla, dark purple-brown in SV with granular contents. Figs. 1A, 2C&D; **basidia** 4-spored, narrowly clavate, in upper part with parallel sides, hyaline, with granular contents especially in SV, 30 - 38 x 6.5 - 8.5 (- 10) µm; sterigmata distinct 2- 4 µm long. Figs. 1D & 2D; **pileipellis** ixodermic with upper gelatinized zone 15 µm thick, suprapellis composed of radially arranged hyphae 4.5 - 6.5 µm broad with shiny walls and aggregated in places to give yellowish brown skeins intermixed with broader elements 13µm plus broad and with some rounded sphaerocytes 20 - 35 µm broad exposed between the skeins; yellowish brown, granular contents in SV in many hyphae but apparently randomly distributed over the pileus except where concentrated in the skeins of hyphae; no prominent dermatocystidia seen, replaced by poorly differentiated obtuse or torpedo-shaped terminal elements 17 - 20 (- 60) x 6.5 -11 µm or mixture of broader elements, generally with granular yellowish sepia contents in SV. Fig. 1E; **stipitipellis** with poorly developed, filamentous, terminal elements, dark purple-brown in SV, seated on hyaline, more-or-less parallel or intertwined, filamentous hyphae 2.5 -3.5 µm broad; similar in structure to parts of the pileipellis but less organized; **clamp connections** absent.

Habitat Thailand, Chacherngsoa Province, Panomsarakam District, Khao Hin Sorn Royal Development Study Area, 22 viii 2004, under *Dipterocarpus alatus*, in plantation, legit Sunadda Yomyart et al., *Wat.* No. 28784 in E; duplicate in Department of Microbiology, Faculty of Science, Chulalongkorn University, Bangkok, Thailand.

Note SV = Sulphovanillin reagent.

Discussion

In overall features the present species closely approaches *Russula cingulata* Buyck & E. Horak, described from Papua New Guinea, but in minutiae there are important differences, especially in that the pileus does not disrupt into flakes or into scales as *R. cingulata*; the basidiospores are also larger and less strongly ornamented. In colour *R. siamensis* resembles members of the North Temperate *Russula amoenolens* Romagn. / *R. sororia* (Fr.) Romell - complex, although the margin of the pileus is pectinate-striate from a much earlier stage probably because of the thinness of the flesh. In the pectinate feature it agrees with *R. pectinatoides* Peck in addition to the slightly russet flush which develops with age at the stipe-base. Buyck & Horak (1999) placed their fungus in Sect. *Fistulosae* subsect. *Meleagrinae* but they indicated that it certainly comes close to Sect. *Ingratae* subsect. *Pectinatineae* erected by Bon (1986). Interestingly *R. amoenolens* is the type of the subsection. It is suggested that *R. siamensis* should be placed therein and this is supported by the ITS sequencing data. Miller & Buyck (2002) examined ribosomal DNA sequences for 87 species of *Russula* from Europe but these did not include an annulate species. Therefore at present available DNA sequences for the ITS region of members of Russulaceae is limited, and only indicative as the family is probably of the order of 400 species and complex in nature. At the moment our novel species sits uneasily until further annulate species are sequenced. The species was introduced as *R. insignis* Quél., then later in the same year it was recombined as a variety of *R. pectinata* – *R. pectinata* var. *insignis* (Quél.) Quél. (Quélet 1888) and considered by Romagnesi (1967) to be the same as *R. livescens* (Batsch) Quél. but differs in that there is evidence of a pale yellow marginal veil. This would be in keeping with a placement near to *R. siamensis* with its rather more fully developed velar tissue.

In parallel to the Thai fungus, *Russula cingulata* is found with a member of the *Dipterocarpaceae* but the host of *R. siamensis* occurs only in the evergreen forests of Myanmar (Burma) and Thailand, and the Andaman Islands. *Dipterocarpus alatus* is not found in Peninsula Malaysia, except doubtful records at 152 m. in Penang and near Ipoh in Perak. (Symington 1941). It would, however, be expected to occur further north but not south into the collecting areas visited by Horak in Papua New Guinea since there appears to be some host specificity shown by the two S.E. Asian annulate *Russula* spp.

Acknowledgements

This work was financial supported by the Thailand Research Fund through the Royal Golden Jubilee Ph.D. program to S. Yomyart and P. Sihanonth under the research project no. 2.B.CU/47/N.1. Thanks to Dr. Warawut Chulalaksananukul for use of his laboratory facilities. We are grateful to Professor Steven Miller and Professor David Pegler for reviewing the manuscript.

Literature Cited

Beeli M. 1928. Contribution à l'étude de la Flore mycologique du Congo, champignons récoltés par Mme. Goossens et determinés par M. Beeli. V. Fungi Goossensiani. Bulletin de la Société Royale de Botanique de Belgique 2nd series 60: 153-169.

Beeli M. 1936. Contribution à l'étude de la Flore mycologique du Congo, champignons récoltés par Mme. Goossens et determinés par M. Beeli. XI Fungi Goossensiani. Bulletin du Jardin Botanique National de Belgique 14: 83-91.

Bon M. 1986. Novitates. Documentes Mycologigues 17 (No 65): 51-56.

Buyck B. 1988a. Étude microscopique de specimens types de Russules tropicales de la sous section *Diversicolores*. Mycotaxon 33: 57-70.

Buyck B. 1988b. ditto. sous section *Pluviales*. Mycotaxon 33: 71-80.

Buyck B. 1993. *Russula* I (*Russulaceae*). Flore Illustrée des Champignons d'Afrique Centrale, (Fasc.15): 337-447.

Buyck B. 1994. *Russula* II. (*Russulaceae*). Flore Illustée des Champignons d'Afrique Centrale (Fasc. 16): 411-541.

Buyck B, Horak E. 1999. New species of *Russula* (Basidiomycotina) associated with *Anisoptera* (*Dipterocarpaceae*) in Papua New Guinea. Australian Systematic Botany 12: 727-742.

Buyck B, Ovrebo CL. 2002. New and interesting *Russula* species from Panama. Mycologia 94: 888-901.

Buyck B, Thoen D, Watling R. 1986. Ectomycorrhizal fungi in Africa, in IJ Alexander et al. (eds.), Essays on the ecology of the Guinea-Congo Rainforest. Royal Society of Edinburgh Proceedings, Series B. 104: 313-334.

Gardes M, Bruns TD. 1993. ITS primers with enhanced specificity for basidiomycetes: application to the identification of mycorrhizae and rusts. Molecular Ecology 2: 113-118.

Heim R. 1938a. Diagnoses latines d'espèces et varietés nouvelles de Lactario-russulés du domaine oriental Madagascar. Candollea 7: 374 -394.

Heim R. 1938b. Les Lactario-russulés du domiane oriental de Madagascar, essai sur la classification et la phylogenie des *Asterosporales*. Prodrome à une flore mycologique de Madagascar et dépendences 1: 196 p. 4 pl.

Heim R. 1948. Phylogeny and classification of macro-fungi. Transactions of the British Mycological Society 30: 161-178.

Heim R. 1971. The interrelationships between the *Agaricales* and *Gasteromycetes*. 505-534, in RH Petersen (ed.), Evolution in the Higher Basidiomycetes. Knoxville, University of Tennessee Press.

Miller SL, Buyck B. 2002. Molecular phylogeny of the genus *Russula* in Europe with a comparison of modern infrageneric classifications. Mycological Research 106: 259-276.

Pegler *DN*. 1983. Agaric Flora of the Lesser Antilles. Kew Bulletin. Additional Series IX: 617-621 pl. 21A-B, figs 126 a-h.

Pegler DN, Singer R. 1980. New Taxa of *Russula* from the Lesser Antilles. Mycotaxon 12: 92-96.

Quélet L. 1888. Flore Mycologique de la France: 346.

Romagnesi H. 1967. Les Russules d'Europe et d'Afrique du Nord. 998pp. Bordas, France.

Singer R. 1986. The *Agaricales* in modern taxonomy. Königstein, Koeltz Scientific Books.

Symington CF. 1941. Forest Manual of Dipterocarps. Malayan Forest Manual 16, 244 pp.

White T, Bruns T, Lee S, Taylor J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. 315-322, in MA Innis et al. (eds), PCR Protocols: a guide to methods and applications. San Diego, Academic Press.

Zhou Z, Miwa M, Hogetsu T. 1999. Analysis of genetic structure of a *Suillus grevillei* population in a *Larix kaempferi* stand by polymorphism of inter-simple sequence repeat (ISSR). New Phytologist 144: 55- 63.

MYCOTAXON

Volume 95, pp. 255–260 January–March 2006

Embellisia oxytropis, a new species isolated from *Oxytropis kansuensis* in China[1]

QI WANG[2]

qwang2003@hotmail.com
Institute of Mycology, Jilin Agricultural University
Changchun 130118, P. R. China

HIDEYUKI NAGAO

Genebank, National Institute of Agrobiological Sciences
Tsukuba 305-8602, Japan

YU-LING LI & HONG-SHENG WANG

Institute of Grassland, Qinghai Academy of Animal Science & Veterinary Medicine
Xining 810003, P. R. China

MAKOTO KAKISHIMA

Graduate School of Life and Environmental Sciences, University of Tsukuba
Ibaraki 305-8572, Japan

Abstract—*Embellisia oxytropis*, isolated from *Oxytropis kansuensis* in China, is described and illustrated as a new species. This species is characterized by cylindrical conidia with 3-4 transverse septa and 1-celled abundant chlamydospores.

Key words—cultures, isolation, conidiophores, conidia, spores

Introduction

Since the genus *Embellisia* was established and segregated from *Helminthosporium* by Simmons (1971) based on the morphological characteristics of conidiophores, conidia and conidial septa, 21 species have been described or transferred from other genera (de Hoog & Muller 1973; Muntañola-Cvetković & Ristanović 1976; Simmons 1983, 1990, 2004; David et al 2000).

---

[1] Supported by National Natural Science Foundation of China (No. 30270008) and Japan Society for the Promotion of Science (JSPS). Contribution no. 203, Laboratory of Plant Parasitic Mycology, Graduate School of Life and Environmental Sciences, University of Tsukuba, Japan.

[2] Corresponding author

Oxytropis kansuensis Bunge (*Leguminosae*) is widely distributed in grassland of Tibet plateau in China. This species is reported as a locoweed, which is poisonous to animals and causes diseases of livestock (Chang et al. 1981). An endophytic fungus was isolated from its healthy flowers, stems and leaves. Based on the morphological examination we consider this fungus a member of *Embellisia*. Comparative morphology with 21 species shows that this fungus is morphologically different from them. Therefore, we describe this fungus as a new species.

Materials and Methods

Plant sample: The healthy locoweed, *Oxytropis kansuensis*, was collected at Mt. Daming, Qinghai, China, July 20, 2004 and kept in a refrigerator at 4C° for fungal isolation.

Isolation: The fungus was isolated with the method modified from Koga (1993) and Braun et al. (2003). Plant samples were first immersed in 50% ethanol for 20s to eliminate bubbles prior to surface sterilization and were sterilized as indicated in Table 1. The sterilized samples were dried on the sterilized filter papers, then cut into small pieces and incubated on PDA medium at 18C° in darkness. After isolation, the fungus was cultured on PDA, CMA, V-8 and EDMA media.

Table 1. Methods of surface sterilization

Solution	Treatment times
70% ethanol	30 seconds
1% NaClO	3 minutes (leaf or flower)
	5 minutes (stem)
Sterilized water	1 minute rinse, 2 times

Examination: The characteristics of the fungal colonies were observed with a stereomicroscope. Hyphae and conidia obtained from cultures were mounted in lacto-phenol solution on glass slides and examined with a light microscope (LM). For scanning electron microscopy (SEM), they were fixed by vapors from 10% glutaraldehyde solution and coated with platinum, and then observed with a Keyence VE-7800 SEM operating at 2kv or 5kv.

Observation of the fungus in plant tissues: The small pieces of plant samples were boiled in the alcoholic lacto-phenol solution (1 part lacto-phenol to 2 parts 95% ethanol) for 5-10min and stained in analine-blue solution for 30sec. Lacto-phenol contains 10g phenol, 10ml glycerine, 10ml lactic acid and 10ml distilled water. After staining they were immersed in the same solution to destain its pigment for a short time. The hyphae in plant tissues were observed with LM by the method of Koga (1993). For SEM observation, small pieces cut from dried plant samples or plant samples whose epidermis was removed were prepared. They were directly coated by platinum with a JEOL JFC-1600 Auto Fine Coater and observed with a Keyence VE-7800 SEM operating at 2kv or 5kv.

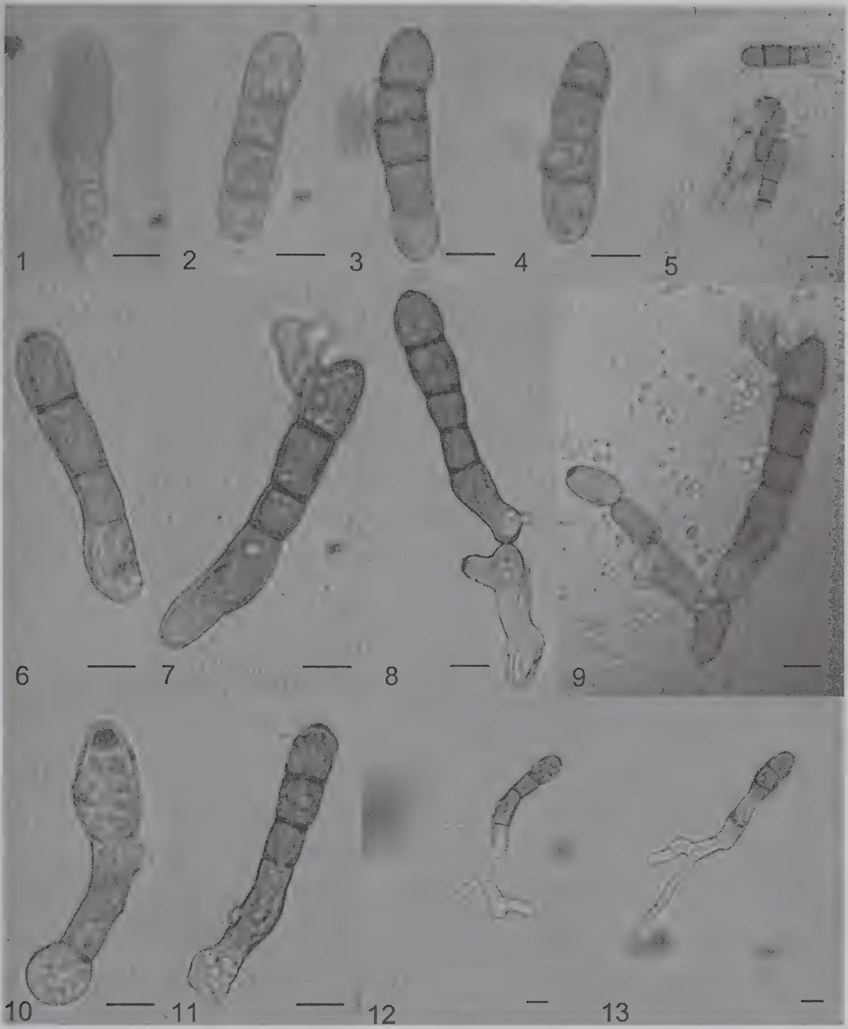


Fig.1. Conidia and conidiophores of *Embellisia oxytropis* isolated from *Oxytropis kansuensis* and cultured on PDA. 1, 2, 3, 4, 6, 7, 10, 11, 12, 13. Conidia. 5, 8, 9. Conidia and conidiophores.

Bars=10μm

Taxonomic description

Embellisia oxytropis Q. Wang, Nagao & Kakish. **sp. nov.** Fig.1

> *Hyphae in PDA effusae pallide brunneae, crassiparietales, septatae, ca. 6~8μm diam. Chlamydosporae abundae, catenulatae, intercalares vel terminales, 1-cellulares, crassiparietales, atrobrunnea. Conidiophora simplicia vel ramosa, pallide brunnea, plerumque geniculata in locis conidiogenis. Conidia longe ellipsoidea vel cylindracea, recta, transverse (2~)3~4-septata, atrobrunnea, 8~10×50~60μm, levia vel minute verruculosa.*

> Holotype: dried culture specimen on PDA, isolated from the leaf of *Oxytropis kansuensis* Bunge, Mt. Daming, Qinghai, China, 20 July 2004, Q. Wang, HMJAU 10012, deposited in the Mycological Herbarium of the Jilin Agricultural University, Changchun, China (HMJAU).
>
> Other specimens examined: dried culture specimens on PDA, isolated from *O. kansuensis*, Mt. Daming, Qinghai, China, July 20, 2004, Q. Wang, HMJAU 10027, 10030, 10032.

Hyphae septate, thick-walled, pale brown, mostly 6~8μm in diam. Chlamydospores abundant as chains, intercalary or terminal, one celled, thick-walled, dark brown. Conidiophores simple or branched, pale brown, mostly becoming several geniculate at conidiogenic loci, secondary sporulation occurring by means of a secondary conidiophore at apex of primary conidia. Conidia long ellipsoid or cylindroid, sometimes straight or slightly inequilateral, mostly with (2~)3~4 transverse septa, dark brown, ca. 8~10×50~60μm. Conidial surfaces smooth or minutely verruculose.

Results and Discussion

Embellisia oxytropis was frequently isolated from healthy plant samples which were kept in a refrigerator at 4C° after collection. The fungus usually appeared from the sides of cross sections 3 days after incubation on PDA. The average ratio of isolation frequency was 20% from leaves, 70% from stems and 85% from flowers, respectively. Therefore, it was suspected that the fungus colonized in all organs of *Oxytropis kansuensis* as an endophyte because no disease symptom was observed in the plant.

Hyphae of the fungus were also frequently observed in the tissues of leaves, stems and flowers with LM and SEM (Fig. 2). They were branched, 2~4μm in diameter and were found under the epidermis or between the plant cells. These hyphae showed the similar characteristics with those observed in cultures on agar media. The hyphae are abundant in flowers and stems but less frequent in leaves. This observation supports the results of fungal isolations. Therefore, we suspected that the flowers were more suitable substrate for the growth of this fungus though it caused no obvious damage to them.

The growth speed on agar media is very slow, about 0.2~0.4mm per day on PDA at 18C°. When the colonies were transferred at 25C° from 18C°, the horizontal growth stopped and the colonies became dark green and formed domes. Colonies on PDA, CMA, V-8 and EDMA showed somewhat different cultural characteristics in color and shape (Fig. 3). On V-8 and PDA, abundant aerial hyphae were observed around the margin of colonies and center of the colonies became dome shaped with dark green to black mycelia. The white flat colonies grew relatively fast on CMA whereas the dark colonies grew more slowly on EDMA.

Embellisia oxytropis is characterized by cylindrical conidia with 3~4 transverse septa and 1-celled abundant chlamydospores. Among 21 described *Embellisia* species, *E. oxytropis* is morphologically similar to *E. abundans* E.G. Simmons, *E. hyacinthi* de Hoog & P.J. Mull. bis, *E. allii* (Campan.) E.G. Simmons, *E. telluster* E.G. Simmons, *E. chlamydospora* (Hoes et al.) E.G. Simmons in shape and/or size of conidia (Simmons 1983, 1990). However, this species is different from them in number of conidial septa and/or direction of septa and also distinguished from *E. hyacinthi* and *E. telluster* by the presence of chlamydospores.

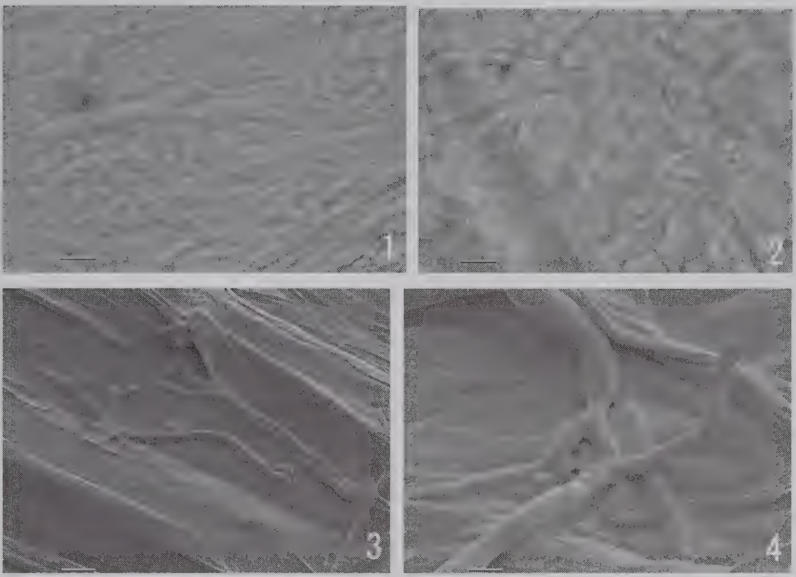


Fig. 2. Hyphae of *Embellisia oxytropis* in plant tissues of *Oxytropis kansuensis* 1. Hyphae observed in the flower by LM. 2. Hyphae observed in the leaf by LM. 3, 4. Hyphae obsetved in the stem by SEM. 1, 2, 3: Bars=10μm, 4: Bar=4μm

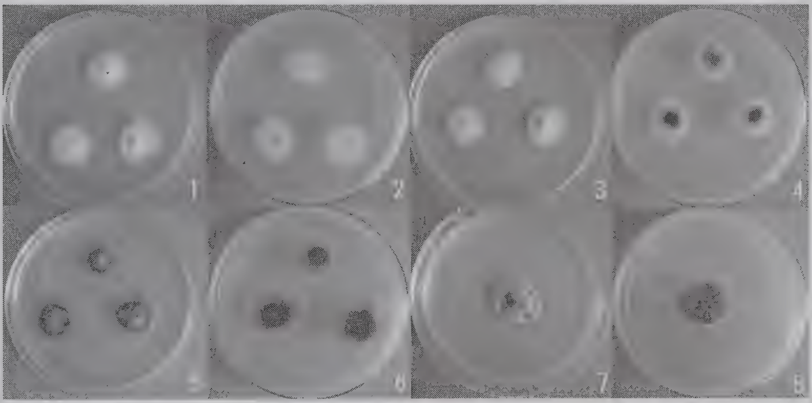


Fig. 3. Colony characteristics of *Embellisia oxytropis* on agar media. 1, 2. On CMA. 3, 4. On V-8. 5, 6. On EDMA. 7, 8. On PDA.

Acknowledgements

Our deep thanks to Dr. K. Okuno and Dr. T. Sato, Genebank of National Institute of Agrobiological Sciences (NIAS), Japan, and Dr. T. Tsukiboshi, National Institute of Floricultural Sciences (NIFS), National Agriculture and Bio-oriented Research Organization, NARO, Japan, for their kind suggestions of our experiments. We are also grateful to Dr. K. Katumoto for his critical reading of the manuscript and revision of the Latin diagnosis, to Dr. Y. L. Guo, Institute of Microbiology, Chinese Academy of Sciences, China, for her critical review of the manuscript.

Literature Cited

Braun K, Romero J, Liddell C, Creamer R. 2003. Production of swainsonine by fungal endophytes of locoweed. Mycol. Res. 107: 980-988.

Chang S, Gao Q, Hou D, Chen J, Zhu X. 1981. Poisoning by *Oxytropis kansuensis*. Acta Veterinaria et Zootechnica Sinica. 12: 145-150 (in Chinese).

David JC, Coles K, Fisher J, Moss ST. 2000. A new species of *Embellisia* from soil with high levels of heavy metals. Mycoscience 41: 533-537.

de Hoog GS, Muller PJ. 1973. A new species of *Embellisia*, associated with skin disease of hyacinths. Neth. J. Pl. Path. 79: 85-93.

Koga H, Christensen MJ, Bennett RT. 1993. Incompatibility of some grass–*Acremonium* endophyte associations. Mycol. Res. 97: 1237-1244.

Muntañola-Cvetković M, Ristanović B. 1976. A new species of *Embellisia* isolated from sea water. Mycologia 68: 47-51.

Simmons EG. 1971. *Helminthosporium allii* as type of a new genus. Mycologia 63: 380-386.

Simmons EG. 1983. An aggregation of *Embellisia* species. Mycotaxon 17: 216-241.

Simmons EG. 1990. *Embellisia* and related teleomorphs. Mycotaxon 38: 251-265.

Simmons EG. 2004. Novel dematiaceous hyphomycetes. Studies in Mycology 50: 109–118.

MYCOTAXON

Volume 95, pp. 261–270 January–March 2006

Some hyphomycetes from Brazil. Two new species of *Brachydesmiella*, two new combinations for *Repetophragma*, and new records

RAFAEL F. CASTAÑEDA RUIZ

rfcastaneda@inifat.co.cu
Instituto de Investigaciones Fundamentales en Agricultura Tropical
"Alejandro de Humboldt", Calle 1 Esq. 2, Santiago de Las Vegas
C. Habana, Cuba. C.P. 17200.

LUÍS FERNANDO PASCHOLATI GUSMÃO

lgusmao@uefs.br
Universidade Estadual de Feira de Santana-UEFS
Departamento de Ciências Biológicas
Caixa Postal 252, 44.031–460, Feira de Santana, BA, Brazil

GABRIELA HEREDIA ABARCA

heredia@ecologia.edu.mx
Instituto de Ecología, A.P. 63, Km,
2.5 Antigua carretera a Coatepec, Xalapa, Veracruz, México

MASATOSHI SAIKAWA

saikawa@u-gakugei.ac.jp
Department of Biology, Tokyo Gakugei University
Nukuikita-machi, Koganei-shi 184–8501, Tokyo, Japan

Abstract—*Brachydesmiella brasiliensis* anam. sp. nov., found on decaying pods of unidentified *Leguminosae* and *Brachydesmiella obclavata* anam. sp. nov., collected on the rotten leaf of an unidentified plant, both from the semi-arid region of Bahia State, Brazil, are described and illustrated. The former is distinguished by navicular to broad fusiform, 3–euseptate, densely verrucose, brown conidia; the latter by obclavate, rostrate, 1–euseptate, pale brown, smooth–walled conidia. A key to *Brachydesmiella* species is provided. *Repetophragma fasciatum* comb. nov. and *Repetophragma filiferum* comb. nov. are proposed. Some other microfungi are reported from the semi-arid region of Brazil.

Key words—anamorph, leaf litter, rotten pod, systematics

Introduction

Over 35 hyphomycetes were collected during mycological surveys of conidial fungi from the semi-arid region in Bahia State, Brazil. Among the collections were two conspicuous fungi clearly related to the genus *Brachydesmiella* G. Arnaud ex S. Hughes (1961) that appear to be new to science. *Brachydesmiella* is characterized by differentiated, mononematous, brown to pale brown conidiophores and tretic, multilocal, sympodial, indeterminate, terminal and intercalary conidiogenous cells. The conidial secession is schizolytic. Six species of *Brachydesmiella* have been validly published in the literature, *B. biseptata* G. Arnaud ex S. Hughes, in Hughes (1961) (the type species); *B. caudata* V. Rao & de Hoog, in Rao & de Hoog (1986); *B. anthostomelloidea* Goh & K. D. Hyde, in Goh & Hyde (1996), *B. orientalis* (V. Rao & de Hoog) Goh, in Sivichai et al. (1998), *B. verrucosa* Goh et al., in Sivichai et al. (1998) and *B. eugecapiellana* R.F. Castañeda et al., in Castañeda Ruiz et al. (2003).

Materials and Methods

Leaf litter samples were placed in separate paper bags and taken to the laboratory. Samples were incubated in Petri dish moist chambers at 25 °C in plastic containers (50 L capacity) with 200 ml of sterile water plus 2 ml of glycerol and examined at regular intervals for the presence of microfungi. Aeration was supplied with a fan (Daytron) for 5 to 10 minutes at 45 minutes intervals. Mounts were prepared in polyvinyl alcohol-glycerol (8.0 g in 100 ml of water, plus 5 ml of glycerol) and measurements made at 1000 x magnification. The conidial fungi were isolated from single conidia picked out under a stereomicroscope after four days incubation from the substrate. They were grown on corn meal agar mixed 1:1 with carrot extract, incubated at 25 °C under alternating cycles of 12 hours of daylight and darkness.

Taxonomy Description

Brachydesmiella brasiliensis R.F. Castañeda, Gusmão & Heredia anam. **sp. nov.**
Fig. (1–5)

> *Ad fungos conidiales, hyphomycetes pertinens. Coloniae in substrato naturali caespitosae, effusae, brunneae. Conidiophora conspicua, mononematosa, erecta, sinuata vel leviter geniculatae, 1– ad 6–septata, 20–110 μm alta, 6–7 μm crassa ad basim, brunnea, levia, simplicia, non ramosa vel interdum ramosa; rami 12–39 μm longi. Cellulae conidiogenae multilocales, treticae, 10–25 × 5–6 μm. Loci conidiogeni pori, 1 μm diam, plerumque obcuri circa poros. Conidia solitaria, navicularia usque ad ample fusiformia, aequilateralia, leviter constricta ad centro, utrimque obtusa vel rotundata cicatricata ad basim, 3–septata, perverrucosa, brunnea, 30–36 × 6–7 μm; 1.5 μm crassa ad basim. Teleomorphosis ignota.*
>
> **Etymology**: Latin, *brasiliensis*, in reference to Brazil.
>
> **Matrix: BRAZIL. BAHIA STATE:** Senhor do Bonfim, *in capsula putrida leguminosa non determinata*, legit L.F.P. Gusmão, 17.VII.2005. **Holotypus:** HUEFS97984. **Isotypus**: INIFAT C05/7.

Conidial fungi, Hyphomycetes. **Colonies** on the natural substratum caespitose, effuse, brown. Mycelium superficial and immersed. Hyphae septate, branched, brown, smooth-

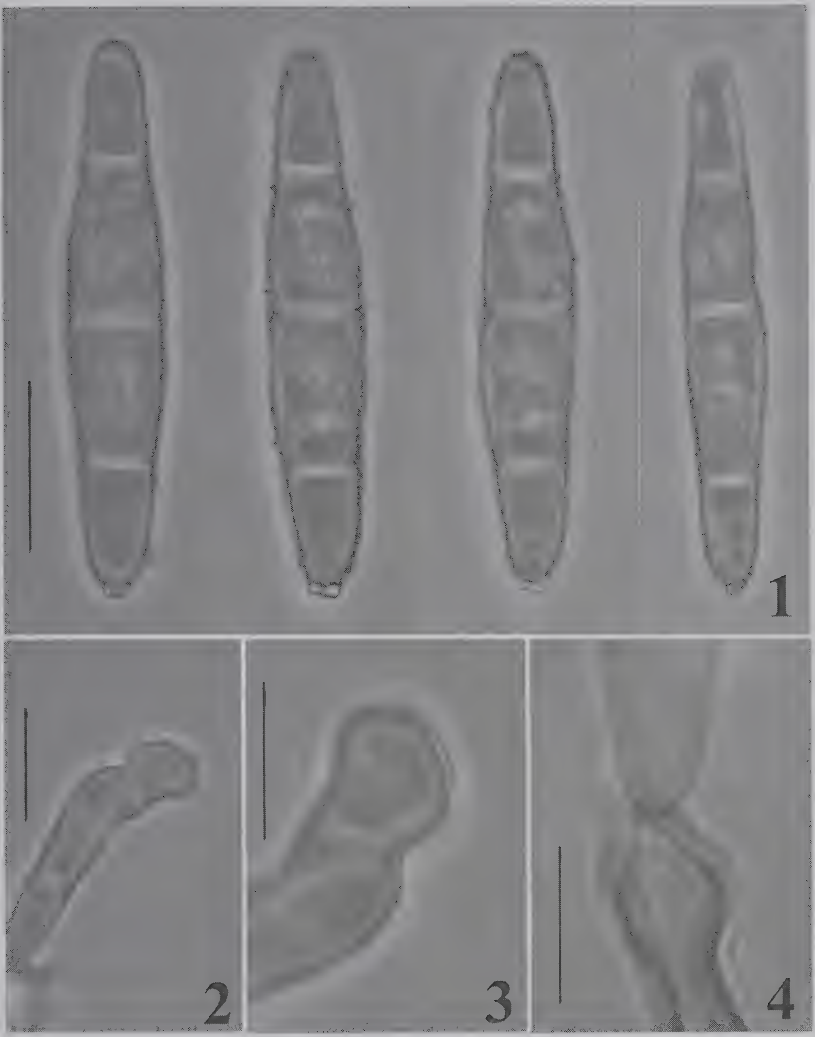


Fig. 1–4. *Brachydesmiella brasiliensis.* 1. Conidia; 2. Geniculate conidiophore; 3–4. Detail of conidiogenous cells. (Bars = 10μm)

walled, 2.0–3.5 μm diam. **Conidiophores** differentiated, mononematous, erect, sinuate to slightly geniculate, somewhat nodose, 1- to 6-septate, 20–110 μm tall, 6–7 μm wide at the base, brown, smooth, mostly simple, sometimes slightly branched; branches 12–39 μm long. **Conidiogenous cells** multilocal, tretic, terminal and intercalary, sympodially proliferating, indeterminate, 10–25 × 5–6 μm, integrated, smooth-walled, brown. **Conidiogenous loci** pores 1 μm diam, with distinct melanized to blackish areas

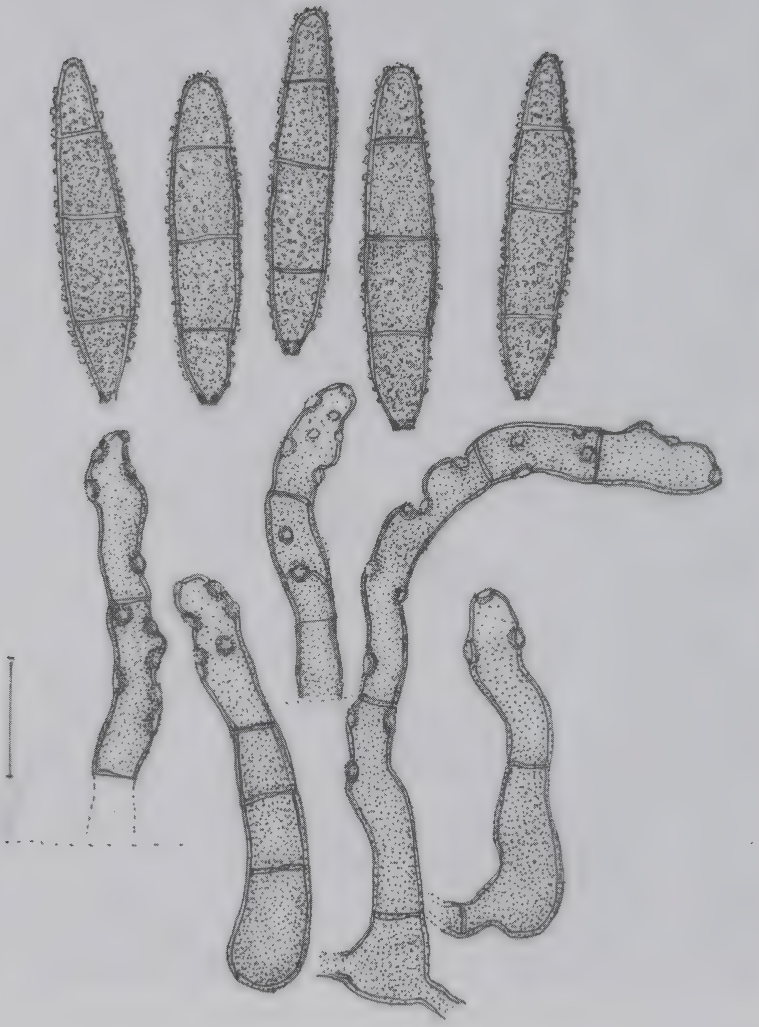


Fig. 5. *Brachydesmiella brasiliensis.* Drawings of conidiophores, conidiogenous cells and conidia. (Bar = 10µm)

around the pores. **Conidial secession** schizolytic. **Conidia** solitary, navicular to broadly fusiform, equilateral, slightly constricted at the middle, obtuse or rounded at the ends, cicatrized at the base with a scar 1.5 µm wide, 3–septate, coarsely verrucose, brown, acropleurogenous, dry, 30–36 × 6–7 µm. Teleomorph: unknown.

Specimen examined: BRAZIL. BAHIA STATE: Senhor do Bonfim, on decaying pods of unidentified *Leguminosae,* L.F.P. Gusmão, 17.VII.2005. **Holotypus:** HUEFS97984. **Isotypus:** INIFAT C05/7.

Note: *Brachydesmiella brasiliensis* resembles *B. verrucosa* and *B. caudata* in conidial ornamentation and number of septa, but *B. verrucosa* has ampulliform, 56– 92 × 12–17 µm, unequally pigmented conidia with pale olivaceous brown central cell that is darker than the apical and basal cells. Also, the apical rostrate cell of *B. verrucosa* is 4–6 µm wide. *Brachydesmiella caudata* is characterized by smooth, ellipsoidal to truncate-limoniform, 52.5–72.0 × 13–33 µm conidia with dark reddish brown central cells.

Brachydesmiella obclavata R.F. Castañeda, Gusmão & Saikawa anam. **sp. nov.**

Figs. (6–10)

Ad fungos conidiales, hyphomycetes pertinens. Coloniae in substrato naturali pilosae usque ad caepitosa, effusae, hypophyllae, brunneae vel luteo-brunneae. Conidiophora conspicua, mononematosa, erecta, sinuata vel geniculata, plerumque nodosa vel inflata ad apicem, 2– ad 5-septata, 40–75 µm alta, 4–5 µm crassa ad basim, brunnea vel dilute brunnea, levia, plerumque simplicia, raro ramosa,; rami 10–22 µm longi. Cellulae conidiogenae multilocales, treticae, 11–20 × 3.0–3.5 µm. Loci conidiogeni pori, 0.5–1 µm diam, plerumque obcuri circumvallati circa pori. Conidia solitaria, obclavata, obscura cicatricata ad basim, 1-septata, raro 2- septata, levia, dilute, brunnea, 18–23 (–26) × 3 µm; 1.5–2 µm crassa ad basim. Teleomorphosis ignota.

Etymology: Latin, *obclavata*, refers to the clavate conidial shape.

Matrix: BRAZIL. BAHIA STATE: Senhor do Bonfim, *in foliis dejectis putridis non determinatae,* legit L.F. P Gusmão, 17.VII.2005. **Holotypus:** HUEFS97983. **Isotypus:** INIFAT C05/14.

Conidial fungi, hyphomycetes. **Colonies** on the natural substratum pilose to caespitose, effuse, hypophyllous, brown to yellow-brown. Mycelium mostly immersed. Hyphae septate, branched, brown to pale brown, smooth-walled, 1.5–2 µm diam. **Conidiophores** differentiated, mononematous, erect, sinuate to slightly geniculate, mostly nodose or inflated towards the apex, 2– to 5–septate, 40–75 µm tall, 4–5 µm wide at the base, brown or pale brown, smooth, mostly simple, sometimes slightly branched; branches 10–22 µm long. **Conidiogenous cells** multilocal, tretic, terminal and intercalary, sympodially proliferating, indeterminate, 11–20 × 3.0–3.5 µm, integrated, smooth-walled, brown. **Conidiogenous loci** pores 0.5–1.0 µm diam, mostly melanized near the pore. **Conidial secession** schizolytic. **Conidia** solitary, obclavate, cicatrized at the base with a scar 1,5 µm wide, 1–septate, rarely 2–septate, smooth-walled, pale brown, acropleurogenous, dry, 18–23 (–26) × 3 µm; 1.5–2 µm at the base. Teleomorph: unknown.

Specimen examined: BRAZIL. BAHIA STATE: Senhor do Bonfim, on dead leaves of unidentified plant, L.F.P. Gusmão, 17.VII.2005. **Holotypus:** HUEFS97983. **Isotypus:** INIFAT C05/14.

Note: *Brachydesmiella obclavata* is somewhat similar to *B. eugecapiellana* in conidial pigmentation, but *B. eugecapiellana* has navicular to narrow fusiform, rostrate, 2–3–septate, 32–40 × 4.0–6.5 µm, verruculose conidia.

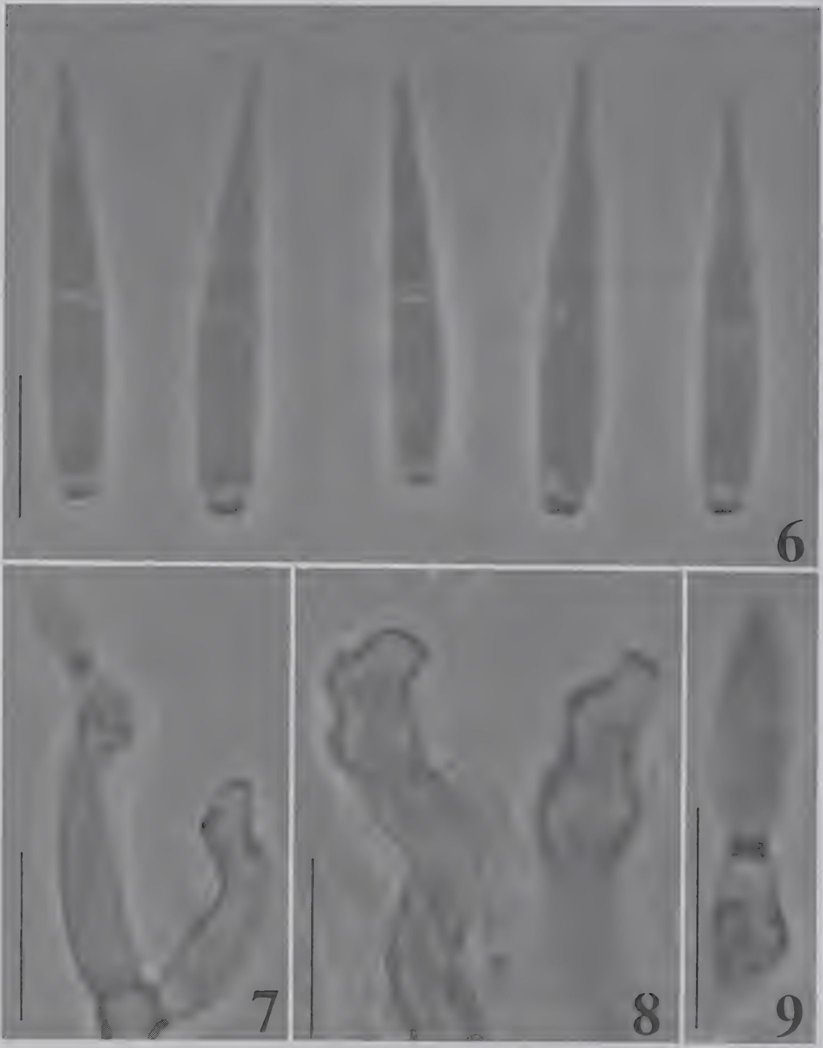


Fig. 6–9. *Brachydesmiella obclavata*. 6. Conidia; 7. Conidiophores; 8. Detail of the conidiogenous cells; 9. Detail of young conidium (Bars = 10µm)

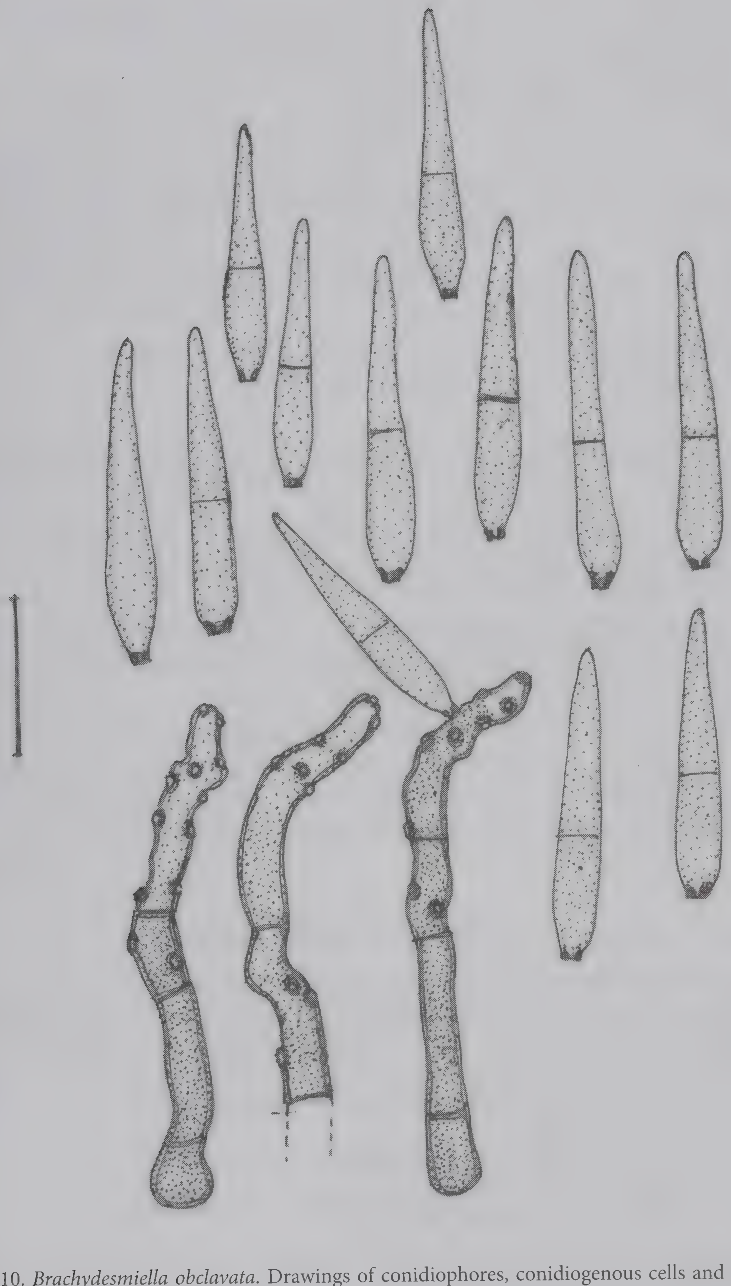


Fig. 10. *Brachydesmiella obclavata*. Drawings of conidiophores, conidiogenous cells and conidia. (Bar = 10μm)

Key to *Brachydesmiella* species

1. Conidia 1–septate 2

1a. Conidia usually with more than 1 septum 3

2. Conidia obclavate, pale brown, 18–23 (–26) × 3 µm; 1.5–2 µm at the base . . . . *B. obclavata*

2a. Conidia limoniform to ampulliform, apical cell cylindrical, pale brown to subhyaline and apical cell darkly olivaceous brown, 35–47 × 14–18 µm . . . . *B. anthostomelloidea*

2b. Conidia pyriform, basal cell broad trapezoid, subhyaline and apical cell blackish brown, 30.0–37.5 × 17.5–22.5 µm . . . . *B. orientalis*

3. Conidia 2–septate 4

3a. Conidia sometimes or always with more than 2 septa 5

4. Conidia limoniform, 37.5–51.0 × 17.5–20.0 µm, unequally pigmented, the apical cell subhyaline, more or less conical, the central cell basal black and obtuse, the basal cell trapezoid, subhyaline . . . . *B. biseptata*

4a. Conidia limoniform-caudate, 52.5–72.5 × 13–33 µm, unequally pigmented, the apical cell subhyaline, cylindrical, the central cell dark reddish brown, elliptical and subhyaline, the basal cell obconical-truncate . . . . *B. caudata*

4b. Conidia ampulliform-caudate, 56–92 × 12–17 µm, unequally pigmented, the apical cell cylindrical subhyaline, the central ellipsoid pale olivaceous brown and hyaline to subhyaline, the basal cell obconical-truncate . . . . *B. verrucosa*

5. Conidia 2– to 3–septate, navicular to narrow fusiform, rostrate, 32–40 × 4.0–6.5 µm, pale brown . . . . *B. eugecapiellana*

5a. Conidia 3–septate, navicular to broadly fusiform, equilateral, slightly constricted at the middle, obtuse or rounded at the ends, 30–36 × 6–7 µm, brown . . . . *B. brasiliensis*

Other microfungi recorded from semi-arid regions in Brazil

Acumispora phragmospora Matsush., Matsushima Mycological Memoirs 1: 3. 1980.

Specimen examined: BRAZIL. BAHIA STATE: Senhor do Bonfim, on rotten stem of *Bambusa* sp., L.F.P. Gusmão, 15.VII.2005 (HUEFS42877; INIFAT C05/10–1).

Diploöspora zinniae Matsush., Matsushima Mycological Memoirs 2: 8. 1981.

Specimen examined: BRAZIL. BAHIA STATE: Senhor do Bonfim, on rotten stem of *Bambusa* sp., L.F.P. Gusmão, 15.VII.2005 (HUEFS42884; INIFAT C05/13)

Phialosporostilbe setosa Bhat & W.B. Kendr., Mycotaxon 49: 57. 1993.

Specimen examined: BRAZIL. BAHIA STATE: Senhor do Bonfim, on rotten stem of *Bambusa* sp., L.F.P. Gusmão, 15.VII.2005 (HUEFS56578; INIFAT C05/9).

Physalidiella matsushimae (R.F. Castañeda & W.B. Kendr.) M. Morelet, Annales de la Société des Sciences Naturelles et d'Archéologie de Toulon et du Var 47: 91. 1995.

Specimen examined: BRAZIL. BAHIA STATE: Senhor do Bonfim, on rotten pod of unidentified *Leguminosae*, L.F.P. Gusmão, 15.VII.2005 (HUEFS56579; INIFAT C05/6).

Repetophragma fasciatum (R.F.Castañeda) R.F. Castañeda, Gusmão & Saikawa **comb. nov.**

Basionym: *Chaetendophragmia fasciata* R. F. Castañeda, in Deuteromycotina de Cuba. Hyphomycetes III. Instituto de Investigaciones Fundamentales Agricultura Tropical, "Alejandro de Humboldt", Cuba p.5. 1985.

=*Endophragmiella fasciata* (R. F. Castañeda) R. F. Castañeda, in Fungi Cubenses III (La Habana, Cuba): 20. 1988.

Specimen examined: BRAZIL. BAHIA STATE: Palmeiras, Capão valley, on decaying leaves of *Cupania paniculata* Cambess. (Sapindaceae), L.F.P. Gusmão, 24.VI. 2000, (HUEFS56675).

Repetophragma filiferum (Piroz.) R. F. Castañeda, Gusmão & Heredia **comb. nov.**

Basionym: *Sporidesmium filiferum* Piroz., in Mycol. Pap. 129: 55. 1972.

Specimen examined: BRAZIL. BAHIA STATE: Palmeiras, Capão valley, on decaying leaves of *Cupania paniculata* Cambess. (Sapindaceae), L.F.P. Gusmão, 24.VI.2000, (HUEFS42798); **BRAZIL. BAHIA STATE:** Senhor do Bonfim, on rotten leaf of unidentified plant, L.F.P. Gusmão, 15.VII.2005, (INIFAT C05/2,).

Note: After re-examination of several samples of both fungi, the schizolytic conidial secession after the production of euseptate conidia by several enteroblastic percurrent proliferations was confirmed; these characters were described for *Repetophragma* Subram. (1992); those fungi were recently recorded in Brazil by Grandi & Silva (2005).

Stylaspergillus laxus B. Sutton, Alcorn & P.J. Fisher, Trans. Br. Mycol. Soc. 79: 340. 1982.

Specimen examined: BRAZIL. BAHIA STATE: Senhor do Bonfim, on rotten pod of unidentified *Leguminosae*, L.F.P. Gusmão, 15.VII.2005, (HUEFS56583; INIFAT C05/6-4).

Acknowledgements

We are grateful to Dr. L. M. Carris (Washington State University), Dr. D. W. Minter (International Mycological Institute) for kindly reviewing the manuscript. We are grateful to the Cuban Ministry of Agriculture for providing facilities during this study. We (RFCR, LFPG and GH) are indebted to the CYTED for support this during this study. The second author extends the grateful to the "PPBIO - semi-arid" and the Brazilian Ministry of Technology & Science (MCT).

Literature Cited

Castañeda Ruiz RF, Kendrick B. 1990. Conidial Fungi from Cuba II. University of Waterloo Biology Series 33: 1-61.

Castañeda Ruiz RF, Iturriaga T, Minter DW, Saikawa M, Vidal G, Velázquez-Noa S. 2003. Microfungi from Venezuela. A new species of *Brachydesmiella*, a new combination, and new records. Mycotaxon 85: 211–229.

Ellis MB. 1971. Dematiaceous Hyphomycetes. Commonwealth Mycological. Institute, Kew.

Goh TK, Hyde KD 1996. *Brachydesmiella anthostomelloidea*, a new species of dematiaceous Hyphomycete from Australia. Mycological Research 100: 1364–1366.

Grandi RAP, Silva TV. 2005. Fungos anamorfos descompositores do folhedo de *Celsalpinia echinata* Lam. In Anais, V Congreso Latinoamericano de Micologia, p. 272, Brasilia, Brazil,

Hughes SJ. 1961. Microfungi. VII. *Brachydesmiella* Arnaud. Canadian Journal of Botany 39: 1095–1097.

Rao V, de Hoog GS. 1986. New or critical hyphomycetes from India. Studies in Mycology. 28: 1–84.

Sivichai S, Goh TK, Hyde KD, Hywel-Jones NL. 1998. The genus *Brachydesmiella* from submerged wood in the tropics, including a new species and new combination. Mycoscience 39: 239–247.

Subramanian CV. (1992). A reassessment of *Sporidesmium* (Hyphomycetes) and some related taxa. Proceedings of the Indian National Science Academy B58: 179–190.

MYCOTAXON

Volume 95, pp. 271–275 January–March 2006

Russula in Himalaya 3: A new species of subgenus *Ingratula*

Kanad Das[1*], J.R. Sharma[2] & N.S. Atri[3]

*daskanad@yahoo.co.in
[1]Mycology and Plant Pathology Group, Agharkar Research Institute
G.G. Agarkar Road, Pune 411004, India

[2]Botanical Survey of India, 192, Kaulagarh Road
Dehradun 248195, India

[3]Botany Department, Punjabi University
Patiala 147002, India

Abstract—*Russula natarajanii,* a new species of subgenus *Ingratula* from Kumaon Himalaya characterized by a whitish pileus, is described and illustrated in detail.

Key words—macrofungi, *Russulaceae*, taxonomy, India

Introduction

Although generally considered one of the largest ectomycorrhizal genera, *Russula* is represented by only approximately 100 taxa on the Indian subcontinent. Of those, only twenty—*Russula delica* Fr., *R. densifolia* Secr. ex Gillet, *R. foetens* Pers., *R. gracillima* Jul. Schäff., *R. heterophylla* (Fr.) Fr., *R. minutula* var. *robusta* Saini et al., *R. nitida* (Pers.) Fr., *R. versicolor* Jul. Schäff., *R. flavida* Frost, *R. compacta* Frost, *R. rhodomelanea* Sarnari, *R. raoultii* Quél., *R. anatina* Romagn., *R. praetervisa* Sarnari, *R. decolorans* (Fr.) Fr., *R. mukteshwarica* K. Das et al., *R. mayawatiana* K. Das et al., *R. puellaris* var. *atrii* K. Das et al., *R. dhakuriana* K. Das et al. and *R. appendiculata* K. Das et al.—have been described in detail from Kumaon Himalaya (Saini et al. 1982, Atri & Saini 1986, Atri et al. 1994, Rawla 2001, Das & Sharma 2001, Das et al. 2002, Das & Sharma 2003, Das & Sharma 2004, Das et al. 2006a, b). Earlier explorations (Atri et al. 1994, Rawla 2001) have been restricted to the other Himalayan regions of Himachal, Kashmir, and Garhwal. Our recent intensive surveys of the different altitudinal zones within Kumaon Himalaya resulted in collections of some additional taxa not previously cited. Comparison with the literature of previously described taxa revealed an undescribed new species, which we formally describe here as *Russula natarajanii.*

Materials and Methods

The present communication is based on specimens collected periodically during recent years from the Pithoragarh and Bageshwar districts of Kumaon Himalaya.

Macromorphological characters were noted from fresh specimens. Colour changes resulting from the addition of $FeSO_4$ on fresh material were also noted. Micromorphological examinations were made of free-hand sections of dried material mounted in 5% KOH, Melzer's reagent, Congo red, Lactophenol-cotton blue, and Carbol Fuchsin. Colour terms follow Kelly & Judd (1955). Microscopic line drawings were made with the aid of a camera lucida at original magnification of 1500x for basidiospores and 1000x for other microstructures. Density of lamellae (L/cm) was measured at the margin of the pileus. Basidiospore measurements exclude the height of ornamentation. Basidium length excludes the length of sterigmata. Quotient (Q = L/W) was calculated considering the mean value of length and width of 25 basidiospores. Herbarium names follow Holmgren et al. (1990).

Description of the species

Russula natarajanii K. Das, J.R. Sharma & Atri **sp. nov.** **Fig. 1**

Etymology: To honour K. Natarajan for his contributions to Indian mycota.

Pileus 60–105 mm latus., planoconvexus, ad umbelicatus, sulcatus, albidus. Lamellae adnexae,,satis distantes, albocremeae. Stipes 40–70 x 16–22 mm, cylindricus, ad subclavatus, albidus. Sporae in cumulo luteoalbae, 6.5–8 x 5.8–7.1 µm, subglobosae ad late ellipticae, amyloideae, subreticulatae. Pleurocystidia 60–90 x 6–10.5 µm, fusiformia. Cheilocystidia nulla. Pileocystidia ad 6 µm lata.

Holotypus: *INDIA, Uttaranchal, Pithoragarh, Dafia Dhura, October 4, 2001, leg. K. Das & J.R. Sharma, KD4097 (**HOLOTYPUS**, BSD).*

Pileus 60–105 mm diam., convex, planoconvex, planoconcave to umbilicate at maturity; pileipellis dry, viscid when moist, white to yellowish white with yellowish gray in the center, sometimes light to medium brown spots at the periphery, unchanging; margin tuberculate-sulcate, decurved, gradually plane at maturity. **Lamellae** adnexed, subdistant to close (ca 6–7 per cm), forked near stipe, brittle, yellowish white to cream; lamellulae absent; edges even. **Stipe** 40–70 x 16–22 mm, dry, central, cylindric to subclavate, concolorous with pileus; context solid to stuffed, white to yellowish white, unchanging. **Taste** mild. **Odor** not distinctive. **Macrochemical** (stipe): $FeSO_4$ positive (salmon). **Spore print** yellowish white.

Basidiospores 6.5–8.8 x 5.8–7.1 µm, subglobose to broadly ellipsoid, Q = 1.08–1.25 (1.34); ornamentation amyloid, up to 0.4 µm high, composed mostly of minute warts and ridges and forming incomplete reticulum. **Basidia** 28–35 x 7.5–9 µm, subclavate to clavate, 4-spored; sterigmata up to 6 µm long. **Pleurocystidia** 60–90 x 6–10.5 µm, emergent up to 30 µm, fusiform with mucronate to capitate apex, content dense. Lamellae edge fertile. **Cheilocystidia** not found. **Subhymenium** layer up to 27 µm thick, cellular. **Pileipellis** composed of suberect hyphae and pileocystidia; pileocystidia up to 5 µm broad. **Stipitipellis** composed of parallel hyphae (up to 3 µm broad). **Stipe trama** composed of numerous sphaerocytes.

Ecology—*Russula natarajanii* grows in close association with *Quercus* species in moist, temperate (2300–2700 m), deciduous/mixed forests in the Kumaon Himalaya.

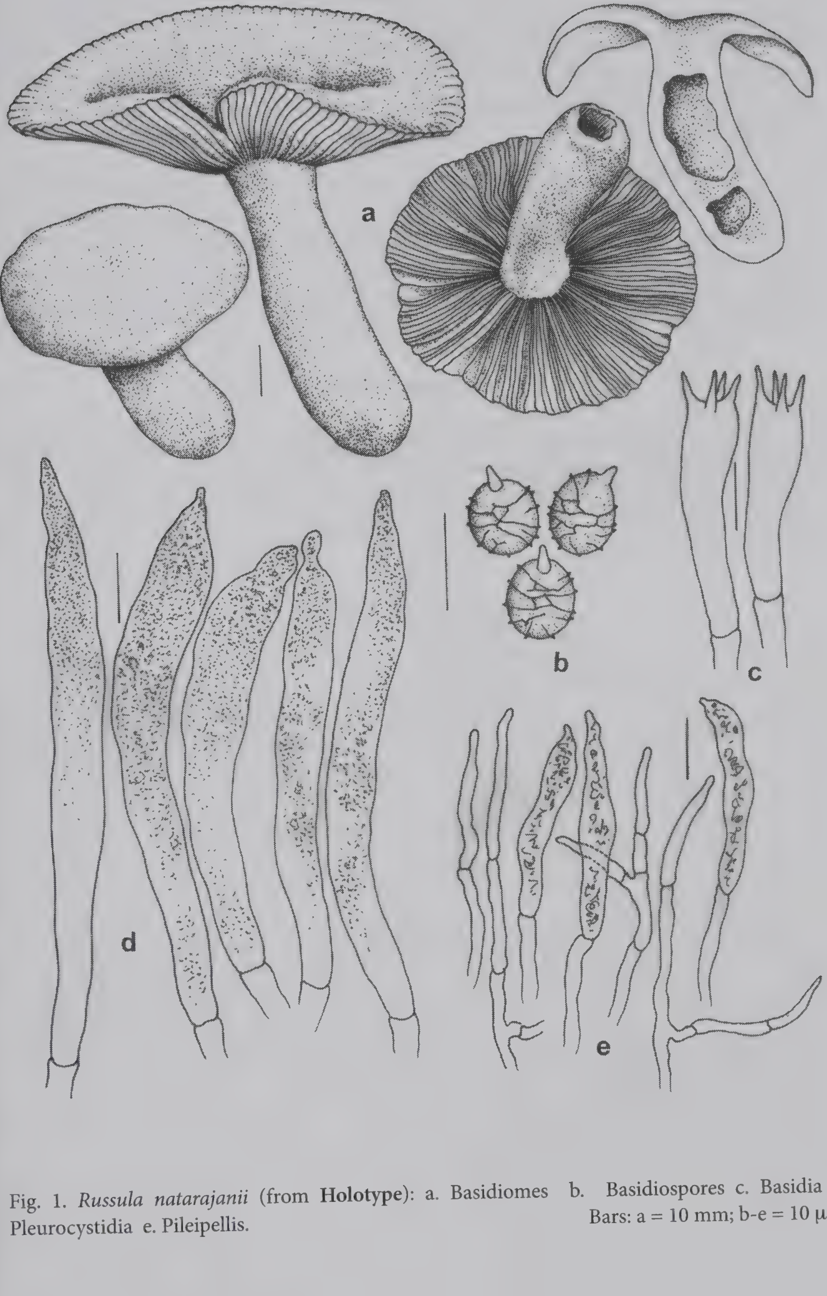


Fig. 1. *Russula natarajanii* (from **Holotype**): a. Basidiomes b. Basidiospores c. Basidia d. Pleurocystidia e. Pileipellis. Bars: a = 10 mm; b-e = 10 μm.

OTHER SPECIMENS EXAMINED—INDIA, **Utaaranchal** BAGESHWAR, Dhakuri, September 2003, leg. K. Das & J.R. Sharma, KD7008 (GUH); *ibid.*, KD7070 (BSD).

Comments—*Russula natarajanii* is distinguished in the field by having the unique combination of a whitish pileus color that is unchanging with bruising or age, a tuberculate pileus margin, and minutely warted basidiospores with ridges. The viscid pileipellis, sulcate pileus margin, adnexed lamellae, low ornamented basidiospores and the presence of pileocystidia support placing *R. natarajanii* in the subgenus *Ingratula* as emended by Sarnari (1998).

In the field, *R. natarajanii* appears quite close to *R. brevipes* Peck var. *brevipes* and *R. delica*. However, typically shorter stipes, numerous lamellulae, absence of tuberculate-sulcate pileus margin (cf. Shaffer 1964, Sarnari 1998), acrid taste, and higher spore ornamentation separate both *R. brevipes* var. *brevipes* and *R. delica* from the newly named species. Moreover, the typically decurrent lamellar attachment also helps to separate *R. brevipes* var. *brevipes* from *R. natarajanii*.

Acknowledgements

We wish to express our gratitude to (Director) Dr. M. Sanjappa and (Joint Director) Dr. D.K. Singh of the Botanical Survey of India, Kolkata and Dr. V.S. Rao, Director, Agharkar Research Institute, Pune for providing facilities. We also thank Jukka Vauras (Åbo Akademi University, Turku, Finland) and Dr. Lorelei L. Norvell (PNW Mycology Service, Portland OR, USA) for critically reviewing the manuscript.

Literature Cited

Atri NS, Saini SS. 1986. Further contribution on the studies of North West Himalayan *Russulaceae.* Geobios New Reports 5: 100–105.

Atri NS, Saini MK, Saini SS. 1994. Indian *Russulaceae* Roze – a check list, in T.A. Sharma, S.S. Saini, M.L. Trivedi & M. Sharma (eds.). 81 – 93. Current Researches in Plant Sciences, Dehradun.

Das K, Sharma JR. 2001. *Russula rhodomelanea* Sarnari – a new record for India. Mushroom Research 10 (2): 109–111.

Das K, Sharma JR. 2003. New records of *Russula* from Kumaon Himalaya. Indian Journal of Forestry 26 (3): 320–326.

Das K, Sharma JR. 2004. *Russula compacta* Frost & Peck – A new record from India. J. Mycol. Pl. Pathol., 34 (1): 149–150.

Das K, Sharma JR, Bhatt RP. 2002. *Russula flavida* Frost – an addition to the Indian ectomycorrhizic fungi. Mushroom Research 11 (1): 9–10.

Das K, Miller SL, Sharma JR. 2006b. *Russula* in Kumaon Himalaya 2: Four new taxa. Mycotaxon 95: 205–215.

Das K, Miller SL, Sharma JR, Sharma P, Bhatt RP. (2005) 2006a. *Russula* in Kumaon Himalaya 1: A new species of subgenus *Amoenula*. Mycotaxon 94: 85–88.

Holmgren PK, Holmgren NH, Barnett LC. 1990. Index Herbariorum. Part 1: Herbaria of the world, 86th ed. Brox: New York Botanical Garden.

Kelly KL, Judd DB. 1955. The ISCC-NBS Method of Designating Colors and a Dictionary of Colour Names. ISCC-NBS Color-Name Charts Illustrated with Centroid Colors. National Bureau of Standards Circular 553. U.S. Government Printing Office, Washington, DC.

Rawla GS. 2001. Himalayan species of *Russula* Pers. Ex S.F. Gray, in P.C. Pande & S.S. Samant (eds.). 1–48. Plant Diversity of the Himalaya. India.

Saini SS, Atri NS, Singer R. 1982. North Indian *Agaricales* – II. Sydowia, Annales Mycologici, Ser. II 35: 238–241.

Sarnari M. 1998. Monografia illustrata del genere *Russula* in Europa. Tomo Primo. Italia. 799 pp.

Shaffer RL. 1962. The subsection *Compactae* of *Russula*. Brittonia 14: 254–284.

MYCOTAXON

Volume 95, pp. 277–280 January–March 2006

Wallemia—a genus newly recorded from China

Guangyu Sun[1], Miao Zhang[1], Rong Zhang[1], Huining Ma[1]
& Mark L. Gleason[2]

sgy@nwsuaf.edu.cn
[1]College of Plant Protection, Northwest A & F University
Yangling, Shaanxi, 712100, China

[2]Department of Plant Pathology, Iowa State University
Ames, Iowa 50011, U.S.A

Abstract—*Wallemia sebi* (Basidiomycota) is reported from China for the first time. The fungus was found on the epicuticular wax of apple fruit sampled from an orchard in Shaanxi Province, China. Its conidiophores are unbranched or sympodial, erect, and phialidic; conidiogenous cells at the apex of conidiophores constrict and disarticulate distally into four arthrospore-like conidia; conidia are one-celled, initially short cylindrical, and finally spherical; the fungus can grow on potato dextrose agar (PDA) and malt extract agar (MEA) media without additional solutes. A description based on the Chinese material and illustrations are provided.

Key words—Wallemiomycetes, taxonomy, sooty blotch

Introduction

Wallemia sebi (Fr.) Arx is a cosmopolitan xerophilic and osmophilic fungus that has been reported from several continents, usually on sweet food (jams, fruits, sugar, cakes), salted food (salted meats, fish, peanuts), dried materials (causing spoilage on dried hay, bread, and dried fruits) (Samson et al. 2002), and seeds (sunflower, rye, wheat), as well as in indoor environments (Takahashi 1997). Terracina (1974) found that the septum of *W. sebi* was similar to those formed by many basidiomycetes and some ascomycetes. Moore (1986, 1996) further ascertained that the hyphae of *W. sebi* contained dolipore septa. Nevertheless *W. sebi* was ascribed to ascomycetes in Kirk et al. (2001). However, Zalar et al. (2005) proposed a new basidiomycetous class, Wallemiomycetes, based on unique dolipore morphology, xerotolerance, and sequence data of ITS rDNA (the rDNA internal transcribed spacer regions), and included three species (*Wallemia sebi*, *W. ichthyophaga* and *W. muriae*) in the now-basidiomycete genus *Wallemia*. In this paper we provide the first description of morphological and colonial characteristics of *W. sebi* collected in China.

Materials and Methods

Apple fruits were collected from Qianyang County in Shaanxi Province. To obtain a pure culture thalli were sampled directly from the apple fruit surface (Sun et al. 2003).

The isolate was cultured on potato dextrose agar (PDA) at 24° in darkness.

The characteristics of the isolate were described and photographed growing on dishes of PDA and malt extract agar (MEA); fungal structures were mounted in lactophenol for microscopic examination. The colony diameter of the isolate was determined on three media (YGA, YGA2, and M40Y) 15 days after inoculation at 24°.

Media: PDA (200 g potato; 20 g dextrose; 15 g agar; 1000 ml distilled water); MEA (20 g Bacto™ malt extract; 15 g agar; 1000 ml distilled water); YGA (10 g yeast extract, 50 g glucose, 4 g K_2HPO_4, 15 g agar, and 1000 ml distilled water); YGA2 (YGA amended with 100 g glucose); M40Y (powdered malt extract 20 g, yeast extract 5 g, sucrose 400 g, agar 15 g, and 1000 ml distilled water).

Taxonomic Description

Wallemia sebi (Fr.) Arx, Gen. Fungi Sporul. Cult.: 166. 1970.
= *Sporendonema sebi* Fr., Syst. Mycol. 3: 435. 1832.

On surface of apple fruit mycelia are dark brown, forming sooty blotch colonies with thick tree-like branched margins. Colonies on plate usually slow growing, variable in shape. On M40Y, forming mounded cones, walnut or gray brown in color, fragments easily dislodged from colonies and forming satellite colonies on the dish, darker color at the colony margins, sometimes secreting light to dark yellowish mucous drops golden to black in color, 16 mm colony diameter after 15 days. Colonies on YGA clumped, walnut or gray brown, compact, slightly mounded in center, spreading deeply into medium, surface usually dry but sometimes secreting tiny, light-colored drops that are initially golden and slowly become brown or black, sometimes white aerial mycelia, 10 mm diameter after 15 days. On YGA2, 13 mm diameter after 15 days, colonies similar to those on YGA, but including easily dislodged fragments leading to scattered satellite colonies.

On PDA, colonies ridged, 6 mm in diameter after 15 days, hyphae hyaline, smooth, thin-walled, septate, mycelia loosely arranged, branched, and 1.5-3.0 µm wide. Conidiophores erect, short, subhyaline, compactly arranged, unbranched, phialidic, smooth-walled, densely arranged. Conidiogenous cells at the apex of conidiophores, cylindrical, not smooth-walled, basauxically extending, constricting basipetally and disarticulating into four arthrospore-like conidia. Conidia one-celled, initially short cylindrical, slightly brown or nearly hyaline, soon becoming spherical, not smooth, thick-walled, brown or pale brown, 1.5-3.0 (-4.0) µm diameter. Conidia forming long or short, straight or bending chains. On MEA, colonies are similar to those on PDA.

HABITAT: In salty environments, on seeds (sunflower, wheat, rye, and soybean), soil, air, sugary foods, hay, fruits, textiles, man, animal and the surface of apple fruit.

Discussion

Three species, *Wallemia sebi*, *W. ichthyophaga*, and *W. muriae*, were accepted by Zalar et al. (2005) in the genus *Wallemia*. They can be distinguished by morphology (conidial size, having or not having sarcina-like structures) and especially growth on media. Both *W. ichthyophaga* and *W. muriae* grow on MEA only when amended with additional solutes. In our experiment, the isolate from the apple fruit surface grew well on media

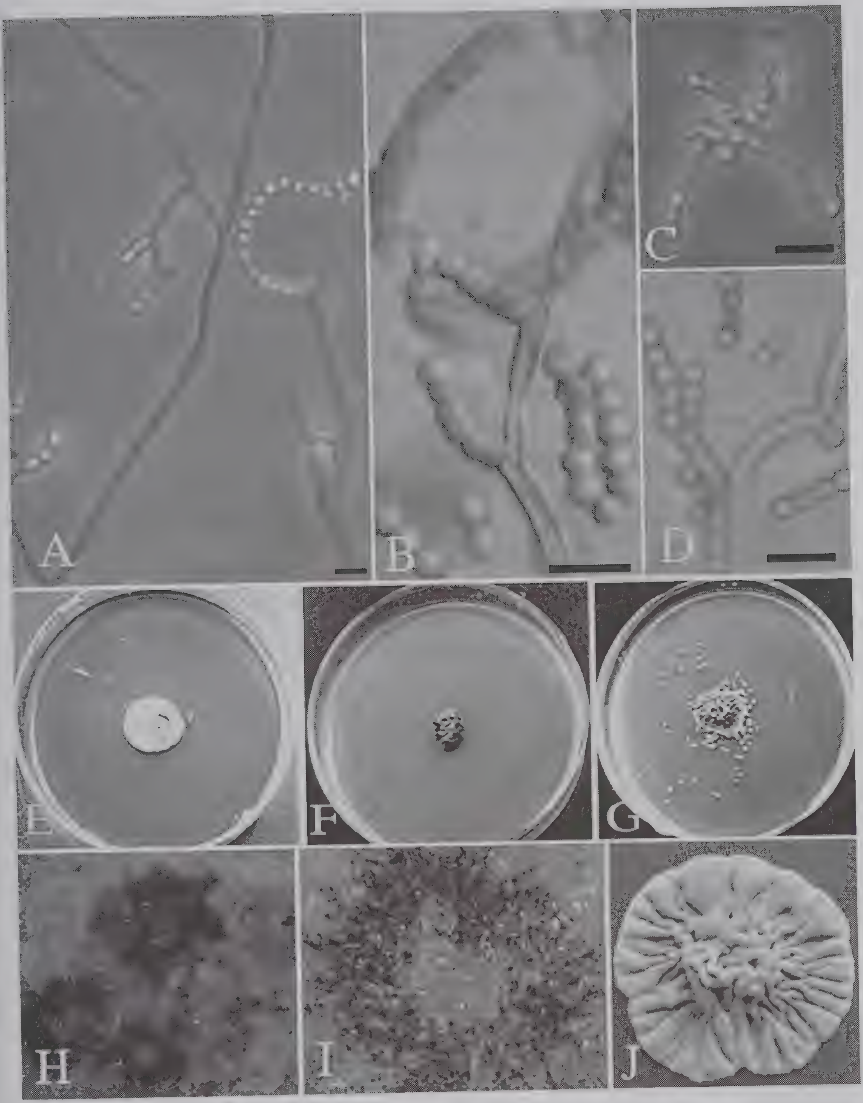


Fig. 1 *Wallemia sebi*

A: Hyphae, conidiophores and chains of conidia; B: Spherical conidia and initially cylindrical conidial chains, and sympodially elongating conidiophores; C: Conidial chains arising from a conidium; D: Conidia in chains; Bars = 10 μm. E: Colonies on M40Y; F: Colony on YGA; G: Colonies on YGA2, satellite colonies scattering in the plate; H: Colony on the surface of apple peel; I: Mycelia of the colony on the apple surface under stereo microscope; J: Colony on PDA.

such as MEA and PDA, its conidial size was similar to that of *W. sebi*, and sarcina-like structures (typical of *W. ichthyophaga*) were absent. Based on these characters we identified the isolate as *W. sebi*.

Wallemia sebi has been reported commonly as growing on food and from the indoor environments. This is the first report of its occurrence as a saprophyte on living plants.

Acknowledgements

This work was supported by International Science Foundation (D/S3538-1). The authors wish to thank Dr. Turner Sutton (Department of Plant Pathology, North Carolina State University, Raleigh, NC 29695) and Dr. Pedro Crous (Centraalbureau voor Schimmelcultures, Fungal Biodiversity Centre, PO Box 85167, 3508 AD Utrecht, The Netherlands) for reviewing the manuscript.

Literature Cited

Kirk PM, Cannon PF, David JC, Stalpers JA. (eds). 2001. Dictionary of the fungi, 9th ed. Wallingford, CAB International.

Moore RT. 1986. A note on *Wallemia sebi*. Antonie van Leeuwenhoek 52: 183-187.

Moore RT. 1996. The dolipore/parenthesome septum in modern taxonomy. 13–35. In: B Sney et al. (eds.), *Rhizoctonia* Species: Taxonomy, Molecular Biology, Ecology, Pathology and Disease Control. Dordrecht, Kluwer Acad. Publ.

Samson RA, Hoekstra ES, Frisvad JC, Filtenborg O. 2002. Introduction to Food and Airborne Fungi, 6th ed. Centraalbureau voor Schimmelcultures, Utrecht, the Netherlands.

Sun GY, Zhang R, Zhang Z, Zhang M. 2003. Isolation of sooty blotch and flyspeck fungi from apple surface by picking up the thalli. Acta Phytopath. Sin. 33 (5): 479-480 [in Chinese].

Takahashi T. 1997. Airborne fungal colony-forming units in outdoor and indoor environments in Yokohama, Japan. Mycopathologia 139 (1): 23-33.

Terracina FC. 1974. Fine structure of the septum in *Wallemia sebi*. Can. J. Bot. 52: 2587-2590.

Zalar P, de Hoog GS, Schroers HJ, Frank JM, Gunde-Cimerman N. 2005. Taxonomy and phylogeny of the xerophilic genus *Wallemia* (Wallemiomycetes and *Wallemiales*, cl. et ord. nov.). Antonie van Leeuwenhoek 87 (4): 311-128.

MYCOTAXON

Volume 95, pp. 281–284 January–March 2006

Phialophora sessilis, a lithobiont fungus

Giuseppe Caretta[1], Solveig Tosi*[1], Eduardo Piontelli[2]
& G.S. de Hoog [3]

solveig.tosi@unipv.it

[1]Sezione di Micologia, Dipartimento di Ecologia del Territorio e degli Ambienti Terrestri
Università di Pavia
via S. Epifanio 14, 27100 Pavia, Italy

[2]Catedra de Micologia, Facultad de Medicina, Universitad de Valparaiso
Casilla 92, Valparaiso, Chile

[3]Centraalbureau voor Schimmelcultures
P.O. Box 85167, NL-3508 AD Utrecht, The Netherlands

Abstract—*Phialophora sessilis* was repeatedly isolated from marble powder in a cemetery site in Pavia (Italy). Morphological characteristics of this strain are reported. Its probable role as a lithobiont with a high adaptability to different organic sources is discussed.

Key words—rock-inhabiting fungi, adelophialide

Introduction

During a survey on rock-inhabiting fungi from marble powder in the cemetery site of Sommo, a suburb of Pavia (Italy), numerous fungal taxa were isolated. Among the species recognized by molecular identification was *Phialophora sessilis* de Hoog (de Hoog et al. 1999). In this paper the first record of this species in the Mediterranean is reported and the isolation of the fungus from nutrient-poor substrates such as marble powder is discussed.

Phialophora sessilis was firstly reported by de Hoog et al. (1999) from *Picea abies* resin in Baarn (Netherlands) and described in a comparative study of 34 strains belonging to the *Phialophora verrucosa* complex. Additional strains of *P. sessilis* originated from forest soil in Sweden, from the lichen *Peltigera polydactyla* and from a biological filter for styrene-containing fumes (de Hoog et al. 1999; for further data refer to www.cbs.knaw.nl/databases/). Important phenetic characteristics of *P. sessilis* are the dark, slow-growing colonies, conspicuous collarettes which are darker than the rest of the phialide and are laterally inserted on undifferentiated hyphae, and conidia inflating to germinating cells prior to germination and then frequently bearing phialidic collarettes. A key to *Phialophora* species occurring as opportunists on humans and bearing morphological similarity to *P. sessilis* was provided by de Hoog et al. (2000).

Phialophora sessilis, despite its apparent phylogenetic position in the Ascomycetes order *Chaetothyriales* containing numerous opportunists on humans (de Hoog et al. 1999), has never been found causing infections in warm- or cold-blooded animals, and it seems to lack any ecological preference. Wrona & Grabowski (2004 a, b) considered *P. sessilis* to be one of the causal agents of apple sooty blotch disease, growing their strain only on the apple skin, utilizing fructose and glucose as primary source of carbon (Wrona & Grabowski 2004 a). However, the possibility is not excluded that a well-known *Phialophora*-like apple-colonizer, *Cadophora malorum* (Kidd & Beaumont) W. Gams (McColloch 1944; Gams 2000) was concerned. We were unable to verify this, as no voucher strain was sent upon request.

The present finding of consistent growth on marble may shed some light on the ecology of *P. sessilis.*

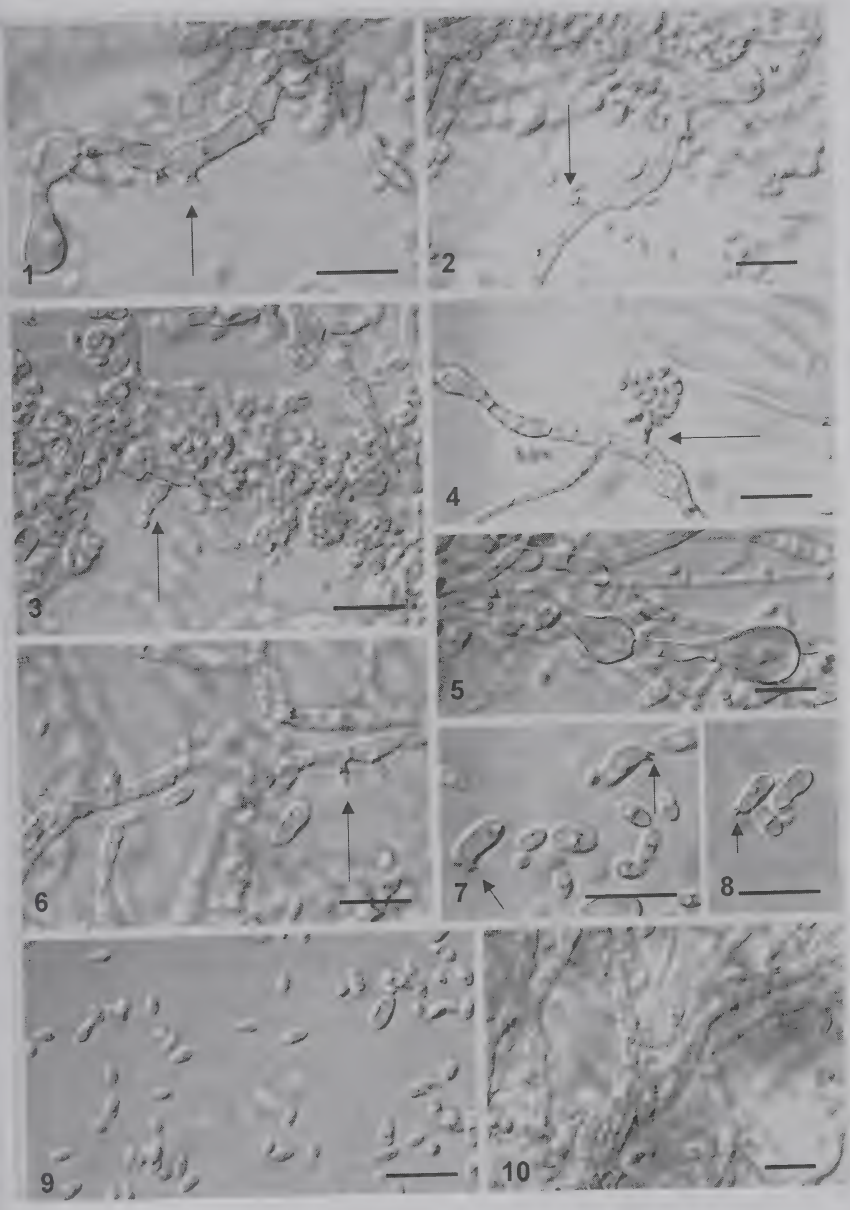
Material and Methods

Powder of marble, originating from an indoor cemetery marble stone near Pavia (Italy), was collected in a sterile plastic box in November 2003. The powder was spread on Petri dishes containing potato dextrose agar (PDA Sigma-Aldrich, Steinheim, Germany), dichloran rose bengal agar (DRBA, Sigma-Aldrich), oat meal agar (OA, Sigma-Aldrich) and potato carrot agar (PCA).

Media were prepared separately with and without cycloheximide, tested at a concentration of 0.1% (w/v). Numerous fungal taxa were isolated and among these *P. sessilis* was, consistenly appearing on all plates and media. Cultural characteristics and morphology were studied on malt extract agar 2% (MEA 2%, ME of Sigma-Aldrich), DRBA, OA, PCA, water agar (WA, bacteriological agar of Sigma-Aldrich) in which cycloheximide, sterile marble powder and sterile $CaCO_3$ were added separately and compared with controls. The plates (in triplicate) were incubated at 20°, 25°, 30°C and observed after 30 days. Tolerance of 5%, 10% NaCl (w/v) was detected in 5% glucose liquid medium at 20°C, following method of Kurtzman & Fell (1998). Radial growth of colonies was measured after 30 days on PDA at 8°, 15°, 20°, 25°C. Morphological observations were made on PDA after 7 weeks of incubation by means of Zeiss microscope (Axioskop2 Plus) connected with a Ks-100 imaging System Release 3:0. Each mean value reported in this paper was calculated from a set of 50 measures.

Results and discussion

Molecular ID using the rDNA Internal Transcribed Spacer (ITS) region resulted in 100% sequence identity with the ex-type strain of *Phialophora sessilis*, CBS 243.85. The morphological characteristics of our specimens of *P. sessilis* isolated from marble powder agree with those described by de Hoog et al. (1999) and can be summarized as follows (Figs 1-10): colonies olivaceous black, attaining up to 20 mm in diam in 30 days on PDA at 20°C and 7 mm in diam at 8°C, granular, cerebriform. Optimum of growth temperature: 20°C. No growth at 30°C. Hyphae torulose near the germinating cell, evenly wide higher up the filament, smooth-walled, 2.25–3.68 μm; sometimes inflated hyphal cells 4.0–9.7 μm wide; most phialides intercalary, with conspicuous, dark collarettes inserted laterally on the supporting cells (adelophialides), 1.4–2.3 × 1.6–2.2 μm. Non-sessile phialides rare, 3.6–9.4 × 2.0–2.8 μm with similar collarettes. Conidia subhaline,



Figs 1-2. Sessile collarettes (arrow). Figs 3-4. Conidia in slimy balls and some conidiogenous cells (arrow). Fig. 5. inflated hyphal cells. Fig. 6. Phialides bearing conidia (arrow). Figs 7-8. Conidia with open collarettes (arrow). Fig. 9. Small conidia. Fig. 10. Aspect of mature mycelium.

Bar in all figures 10 µm.

smooth, biguttulate, obovoidal to ellipsoidal, of two types: (1) 2.3–4.1 × 1.5–2.2 µm, and (2) 4.7–7.0 × 2.4–3.5 µm. Conidia in slimy heads 6.2–8.8 × 7.1–8.2 µm. Chlamydospores absent.

Little variation was observed in *P. sessilis* growing on the different cultural media used. PDA was the most suitable substrate for growth. Differences in tolerance of NaCl and in the growth temperature were recorded. The strain isolated from marble can grow well with 5% NaCl and weakly with 10% NaCl. It grows at relatively low temperature reaching 7 mm in diam at 8°C in one month and does not grow at 30°C.

Interest in *P. sessilis* is driven particularly by the apparent capacity of this fungal species to colonize and survive in lithic material such as marble, by the variety of sources of isolation not easy degradable (resin, styrene, lichen) and by the low competitive ability with mesotrophic microorganisms. Colonization of marble, where it probably lives on degraded bacteria or components of air pollution (Sterflinger & Prillinger 2002) then would fit such ecology. Prenafeta-Boldú et al. (2006) suggested that the assimilation of toxic hydrocarbons might be an essential factor in the ecology of black yeast-like members of the *Chaetothyriales*. The detection and identification of this possible lithobiont is important for the future studies on the deteriogenic processes of stone monuments where chemioorganotrophic bacteria, fungi, and phototrophic organisms, are present in microbial consortium. The finding of *P. sessilis*, consistently isolated at the sampling site in Pavia (northern Italy), extends southward the distribution area and habitat of this rare species, isolated to central and northern Europe.

Acknowledgments

We are grateful to Prof. S. Onofri (Dipartimento di Scienze Ambientali, Università della Tuscia, Viterbo, Italy) and Prof. C. Urzì (Dipartimento di Scienze Microbiologiche, Genetiche e Molecolari, Università di Messina, Italy) for presubmittal review of the manuscript.

References

de Hoog GS, Weenink XO, Gerrits van den Ende AHG. 1999. Taxonomy of the *Phialophora verrucosa* complex with the description of two new species. Stud. Mycol. 43: 107-122.

de Hoog GS, Mayser P, Haase G, Horré R, Horrevorts AM. 2000. A new species, *Phialophora europaea*, causing superficial infection in humans. Mycoses 43: 409-416.

Gams W. 2000. *Phialophora* and some similar morphologically little-differentiated anamorphs of divergent ascomycetes. Stud. Mycol. 45: 187-199.

Kurtzman CP, Fell JW. 1998. The Yeasts: a taxonomic study. Elsevier, Amsterdam, The Netherlands.

McColloch LP. 1944. A study of the apple rot fungus *Phialophora malorum*. Mycologia 36: 576-590.

Prenafeta-Boldú FX., Summerbell RC, de Hoog GS. 2006 Fungi growing on aromatic hydrocarbons: biotechnology's unexpected encounter with biohazard. FEMS Microbiol. Rev. (in press).

Sterflinger K, Prillinger H. 2001. Molecular taxonomy and biodiversity of rock fungal communities in an urban environment (Vienna, Austria). Antonie van Leeuwenhoek 80: 275–286.

Wrona B, Grabowski M. 2004 a. Influence of fructose and glucose occurring on fruit surface on the growth of fungi that cause sooty blotch of apple. J. Plant Protection Res. 44 (4): 287-291.

Wrona B, Grabowski M. 2004 b. Etiology of apple sooty blotch in Poland. J. Plant Protection Res. 44 (4): 294-297.

MYCOTAXON

Volume 95, pp. 285–293 January–March 2006

Revision of *Termitomyces* species originally described from China

B.-H. Tang[1,2] T.-Z. Wei[1] & Y.-J. Yao[1*]

yaoyj@sun.im.ac.cn
[1]Key Laboratory of Systematic Mycology and Lichenology
Institute of Microbiology, Chinese Academy of Sciences
Beijing 100080, China
[2]Graduate School of Chinese Academy of Sciences
Beijing 100080, China

Abstract—Revision of the four *Termitomyces* species originally described from China was carried out to clarify their taxonomic status. Among them, *T. bulborhizus* was reported recently with well supported specimens and is confirmed as a distinct and reliable species, whilst the other three, viz. *T. albiceps*, *T. cylindricus* and *T. macrocarpus*, were described in the 1980s and are determined here to be synonymous with other species of the genus. *Termitomyces albiceps* and *T. macrocarpus* are synonyms of *T. eurhizus*, and *T. cylindricus* of *T. aurantiacus*. Full descriptions of available specimens cited in the original publication of *T. albiceps* and *T. cylindricus* are also provided for reference.

Key words—*Tricholomataceae*, termite symbionts, nomenclature, taxonomy

Introduction

Four species of *Termitomyces* R. Heim, an agaric genus associated with termites, have been described from China. They are *T. albiceps*, *T. bulborhizus* T. Z. Wei et al., *T. cylindricus* and *T. macrocarpus*. Among them, *T. bulborhizus* was described recently with descriptions in both English and Latin, well supported by a number of specimens preserved in internationally accessible herbaria (Wei et al. 2004), whilst the other three were published in the 1980s in Chinese with Latin diagnoses and with specimens mostly not easily accessible (He 1985, Zhang & Ruan 1986). Both *T. albiceps* and *T. cylindricus* were described from collections made in Guizhou (He 1985) and were included in Wu (1990) and Ying & Zang (1994). *Termitomyces albiceps* was further included in Zhang (1991) and *T. cylindricus* in Huang (1998). The latter was also reported from Fujian (Guo 1995) and other localities of Guizhou (Hu et al. 2000). However, none of the later publications noted any additional material of the taxa reported. Based on

---

[*] Author for correspondence

the Latin diagnosis of He (1985), Pegler & Vanhaecke (1994) suspected *T. albiceps* was conspecific with *T. eurhizus* or with *T. globulus* R. Heim & Gooss.-Font., and treated *T. cylindricus* as a distinct species. However, according to the full description in Chinese (He 1985), *T. albiceps* is quite different from *T. globulus* in having a broad and blunt perforatorium and blackish brown pseudorhiza in contrast with a scarcely developed (or absent) perforatorium and tawny to rusty brown pseudorhiza in the latter (see Pegler & Vanhaecke 1994). *Termitomyces cylindricus* is medium-sized with pileus 6.5–12.0 cm diam. and with white pseudorhiza, although it was considered similar to *T. globulus* (pileus 8–20 cm diam., tawny to rusty brown pseudorhiza, see Pegler & Vanhaecke 1994) by He (1985). *Termitomyces macrocarpus* was introduced based on two collections from Yunnan (Zhang & Ruan 1986) and was included by Ying & Zang (1994), Zang et al. (1996) and Shao & Xiang (1997). Again, no further collections were noted in these later publications.

In a survey of *Termitomyces* species in China, all the reported taxa of the genus have been examined based on specimen and literature study. Some specimens of *T. albiceps* and *T. cylindricus* cited by He (1985) were traced in the Herbarium Mycologicum, Academia Sinica (HMAS) and the Herbarium of Cryptogams, Kunming Institute of Botany, Academia Sinica (HKAS). They were examined and redescribed in detail and compared with collections of *T. eurhizus* and *T. aurantiacus* from China and abroad to determine their identity. The authors have contacted Mr. Z.-F. Zhang (co-author of Zhang & Ruan 1986), and according to him, the specimens of *T. macrocarpus* are no longer traceable and apparently lost (Zhang, pers. comm. 2003–2004) and study on its taxonomic status has been based on the original description and the follow-up reports. *Termitomyces bulborhizus* is confirmed as a distinct and reliable species in the genus and is not repeated here.

Materials and Methods

Dried specimens from Guizhou in the herbaria listed above were examined both macroscopically and microscopically. The dried herbarium specimens were photographed and the following description is based on the examination of the material. For microscopic studies, free-hand sections of dried basidiocarps, including lamellae, cutis and pileal context, were prepared using a razor-blade and mounted in a 5 % KOH solution. Size ranges of basidiospores, basidia, hyphae of lamella and trama, pileal and stipe context were measured using an ocular micrometer. At least 30 basidiospores and 20 basidia of each mature specimen were measured.

Taxonomy

Termitomyces albiceps S. C. He in Acta Mycol. Sinica 4: 106 (1985). **Fig. 1**

Synonym of ***T. eurhizus*** (Berk.) R. Heim in Arch. Mus. Hist. Nat. Paris, Sér. 6, 18: 140 (1942).

Pileus up to 9.5 cm. diam., applanate with a round perforatorium; surface brown at centre, yellowish brown elsewhere and paling toward margin, radially striate; margin straight, radially splitting. *Lamellae* free, up to 6.0 mm wide, greyish white; crowded, with lamellulae. *Stipe* 9.0 cm long, 1.5 cm thick, central, cylindrical and thickening slightly at ground level; surface greyish to brownish, smooth; solid and fibrous, of longitudinally parallel hyphae, thin-walled and hyaline, 2.5–25 µm diam. *Pseudorhiza* up to 20 cm long, tapering, with a pale yellowish disk connected with termite comb; surface dark brown in upper part and black below, longitudinally striate; solid, fibrous, consisting of thin-walled and hyaline hyphae, 2.5–33 µm diam. *Partial veil* not found. *Context* fleshy, white, of inflated, thin-walled and hyaline hyphae, 2.0–7.5 µm diam., inflating up to 35 µm. *Basidiospores* 5.5–9.0 × 4.0–5.5 µm, ovoid to ellipsoid, thin-walled and subhyaline. *Basidia* 16.0–26 × 6.0–9.0 µm, clavate, tetrasporic, thin-walled and subhyaline. *Lamella-edge* heterogeneous. *Cheilocystidia* 14.0–33 × 10.0–17.0 µm, clavate to pyriform, thin-walled and hyaline. *Pleurocystidia* not found. *Hymenophoral trama* regular, up to 50 µm wide, of thin-walled and hyaline hyphae, 4.0–20 µm diam. *Subhymenial layer* 5.0–10.0 µm wide, of branched, thin-walled and hyaline hyphae, 2.0–6.0 µm diam. *Pileipellis* a repent epicutis of narrow, radially parallel hyphae, 3.0–5.0 µm diam.

Specimens examined - **CHINA: Guizhou:** Xingyi, Baiwayao, alt. 1530 m, solitary on nest of *Odontotermes formosanus* (Shiraki) in coniferous forest, 22 Aug. 1983, S.-C. He 1056, HMAS 47850 (paratype); same details, 26 Aug. 1983, S.-C. He 469, HKAS 14660 (paratype).

Most of the 10 specimens cited by He (1985) in the original description of *T. albiceps*, including the type, are now apparently lost. The above description is based on the two dried collections from Guizhou cited here, which are paratypes of *T. albiceps*. The following specimens of *T. eurhizus* were also examined for comparison to confirm the above determination.

CHINA: Yunnan: Xishuangbanna, Mengla, Menglun, Xishuangbanna Tropical Botanical Garden, on termite nest of *Odontotermes* sp., 1 Aug. 2004, M. Li & B.-H. Tang, T0453, HMAS96507. **Sichuan:** Dechang, Badong Town, Songbai Village, 14 Aug. 2003, H. Deng, Y.-J. Yao, S.-Z. Fu and L. Jiao, W03-36, HMAS 79897; Hongqi Village, 14 Aug. 2003, H. Deng, Y.-J. Yao, S.-Z. Fu and L. Jiao, W03-37, HMAS 84715; Pujiang, purchased in local market, 18 Aug. 2002, B. Wang 200235, HMAS 76913. **INDIA: Kerala:** Malappuram Dist., Calicut University Campus, near school ground, solitary on ground, 30 Jul 1986, V. Vrinda, V 236, F1095161; Malappuram Dist., Botanical Garden, Calicut University Campus, ca. 40-50 m., roadside, front of garden, on ground, 13 Jun 1984, K.M. Leelavathy, F 177, F 1091313. **South Africa: Pretoria**: attached to termite nest underground, I. Bredenkamp, 14 March 1966, PREM 43147.

According to the original description of He (1985), the pileus of *T. albiceps* is up to 18 cm diam., and the surface of its pseudorhiza is blackish brown, similar to that of *T. eurhizus*. Comparison of the above two collections, cited for *T. albiceps* by He (1985), with *T. eurhizus* collections from China and abroad revealed that the pseudorhiza of *T. albiceps* specimens was black, which is a distinct character of the *T. eurhizus* specimens examined. Furthermore, the other characters of the two specimens, such as large basidiocarp, brownish pileus, round perforatorium and size of basidiospores, are almost identical with those of *T. eurhizus* (see Pegler & Vanhaecke 1994). He (1985) mentioned *T. albiceps* had some irregular tubercles on the pileus margin and its stipe surface was covered by some fluffy squamules. However, neither of the two characters was detected on the dried material, and no tubercles could be seen in the illustration of the type in the protologue. Moreover, the present authors consider these two characters are not constant for species identification. The tubercles on the pileus margin may be accidental variation, which can also be found in some *T. eurhizus* collections, e.g. HMAS 96507. The squamules on the stipe, which are occasionally found in some fresh material of *T. eurhizus*, e.g. HMAS 79897, HMAS 84715 and HMAS 96507, are probably ephemeral remains of a partial veil that are easily lost in specimen preservation. According to He (1985), no pleurocystidia were found in *T. albiceps*. This was confirmed in the present study of the two specimens examined. The number of pleurocystidia can vary from numerous to scattered or rare, e.g. *T. clypeatus* R. Heim and *T. entolomoides* R. Heim (Pegler & Vanhaecke 1994, Wei et al. 2003), or absent, e.g. *T. radicatus* Natarajan (Natarajan 1977) in some species of the genus. This phenomenon was also present in some *T. eurhizus* collections, e.g. pleurocystidia were reported rare in AMH 4479 (Sathe & Deshpande 1981) and AMH 4546 (Sathe & Daniel 1981, Tang et al. 2006), and were absent in some specimens examined in this study, e.g. HMAS 76913 and F 1095161. Therefore, the pleurocystidia are also not a constant character for species identification. Consequently, there is no reliable morphological character to distinguish *T. albiceps* from *T. eurhizus* and they are evidently conspecific.

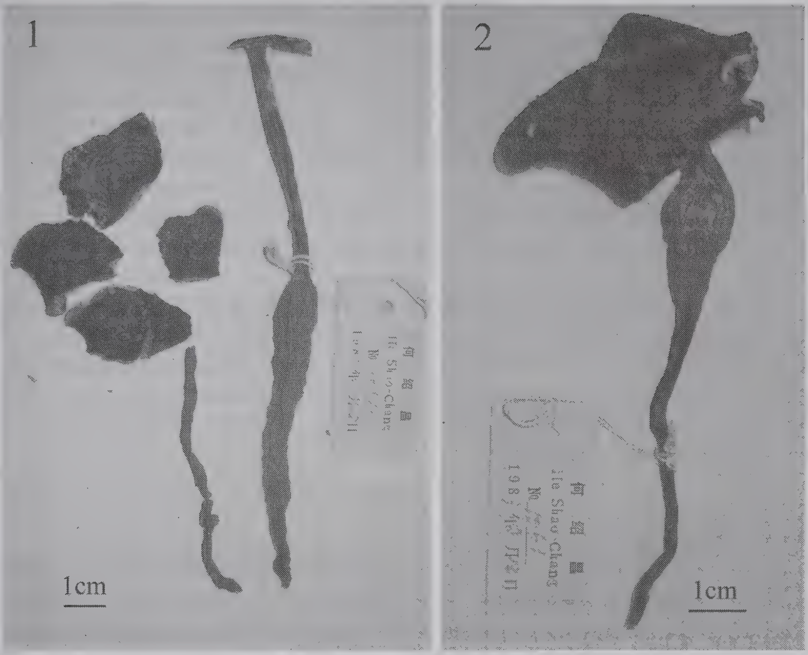
Collections of *Termitomyces albiceps* were found on nests of *Odontotermes formosanus*, *O. quinquedentatus* and *O. periformosanus* (He 1985). In our field work in the southwest of China, *T. eurhizus* was often found associated with *Odontotermes* species.

Termitomyces cylindricus S.C. He in Acta Mycol. Sinica 4: 104 (1985). **Fig. 2**

Synonym of ***T. aurantiacus*** (R. Heim) R. Heim in Termites et Champignons (Paris): 56 (1977).

Pileus up to 9.3 cm diam., conical-applanate, with a small and bluntly pointed perforatoium; surface brown to blackish and darker at the centre, glabrous; margin straight, radially striate and splitting. *Lamellae* free, 3.0–4.0 mm wide; greyish white; crowded, with lamellulae. *Stipe* 6.0–7.0 × 1.5–2.3 cm, central, cylindrical and slightly thickened at ground level; surface greyish white to brownish, smooth and glabrous; solid, of longitudinally parallel, thin-walled hyphae, 2.0–25 μm diam. *Pseudorhiza* up to 17.0 cm, 1.1 cm wide and tapering downward from ground level, terminating with a pale, cylindrical base; surface brownish, longitudinally striate; solid, of longitudinally parallel, thin-walled hyphae, 2.0–25 μm diam. *Partial veil* not found. *Context* fleshy, white, of inflated, thin-walled and hyaline hyphae, normally 2.5–8.0 μm diam. inflating to 35 μm diam. *Basidiospores* 5.0–7.5 × 3.5–4.5 μm, ovoid to ellipsoid, thin-walled and subhyaline. *Basidia* 17.0–22 × 6.0–7.5 μm, clavate, tetrasporic, thin-walled and subhyaline. *Lamellae edge* heterogeneous. *Cheilocystidia* 25–43 × 12.0–23 μm, clavate to pyriform, thin-walled and hyaline. *Pleurocystidia* not found. *Hymenophoral trama* regular, 40–50 μm wide, of thin-walled and hyaline hyphae, 4.0–18.0 μm diam. *Subhymenial layer* narrow, of branched hyphae, 2.0–5.0 μm diam. *Pileipellis* an epicutis of narrow, radial hyphae, containing yellowish granules, 2.5–5.0 μm diam.

Specimens examined - **CHINA: Guizhou:** Xingyi, Baiwayao Town, alt. 1530 m, symbiotic with *Macrotermes orthognathus* Ping et Xu, 22 Aug. 1983, S.-C. He 1062, HKAS 14695 (paratype); alt. 1330 m, 22 Aug. 1983, S.-C. He 1061, HMAS 47851 (paratype).



Figs 1–2. Photographes of *Termitomyces* spp. from China. Fig. 1. Habit of *Termitomyces albiceps* (HMAS 47850). Fig. 2. Habit of *Termitomyces cylindricus* (HMAS 47851).

The above description is solely based on these two dried collections from Guizhou, which were cited in the protologue of *T. cylindricus* (He 1985). The following specimens of *T. aurantiacus* were also examined for comparison.

> **CHINA: Yunnan:** Mengla, Menglun, Xishuangbanna Tropical Botanic Garden, on termite nest, G.-R. Hu & T.-Z. Wei, 8 Aug. 2003, w03-19, HMAS 84720; the same locality, on termite nest of *Macrotermes* sp, M. Li & B.-H. Tang, 2. Aug. 2004, T0456, HMAS 99569; the same locality, on mounds of *Macrotermes* sp., Li & B.-H. Tang, 8 Aug. 2004, T0493, HMAS 99570; Jingdong, Phoenix Mountain, alt. 1260 m, 25 Aug. 1991, Z.-L. Yang 1642, HKAS 23955. **THAILAND: River Kwai:** Kanchanaburi, Kang Chaw, Boonthungs Farm, 20 Sept. 1980, B. J. Bels, K(M) 94661.

The type and most of the 11 specimens cited for *T. cylindricus* by He (1985) cannot now be traced. The two collections cited in the protologue and examined here resemble *T. aurantiacus* in having medium-sized basidiocarps with a pointed perforatorium and pale pseudorhiza. Microscopically, the two *T. cylindricus* specimens are identical with those of *T. aurantiacus*. According to He (1985), *T. cylindricus* can be distinguished from other species of the genus by its cylindrical pseudorhiza base. However, several collections of *T. aurantiacus* from Xishuangbanna, Yunnan, (e.g. HMAS 99569, HMAS 99570 and HMAS 84720), also have a cylindrical base at the end of the pseudorhiza. The present authors found no small squamules mentioned by He (1985) on the stipe surface of the two collections. The structure is probably formed by ephemeral remains of partial veil and can often be seen in fresh material of *T. aurantiacus.*

Termitomyces aurantiacus is distinct in its constantly bright reddish ochraceous to orange pileus and its firm texture (Pegler & Vanhaecke 1994). The pileus of *T. cylindricus* was described as 'brunneolus vel cinerascens' in Latin and 'pale brown to ash-grey' (literary translation) in Chinese by He (1985), and is brown to blackish in the two dried herbarium specimens examined by the present authors, but the illustration of the type material in the protologue (Plate I-2) shows no clear difference in pileus color from *T. aurantiacus* as seen in the field, although the plate seems not to be produced perfectly in color.

He (1985) described the lower part of the pseudorhiza of *T. cylindricus* as 'pale yellowish green', different from the white pseudorhiza of *T. aurantiacus* (Pegler & Vanhaecke 1994). Unfortunately, this character cannot be detected in the dried specimens. In some *T. aurantiacus* collections from Yunnan, the lower part of the pseudorhiza was slightly yellowish when fresh, but no green pseudorhiza have been seen in any species of *Termitomyces.*

Based on the discussion above and the morphological characters observed from the two specimens of *T. cylindricus*, it is concluded here that *T. cylindricus* is synonymous with *T. aurantiacus.*

Termitomyces aurantiacus is widely distributed in both Africa (Heim 1977, Mossebo et al. 2002, Aanen et al. 2002, Frøslev et al. 2003) and Asia (Pegler & Vanhaecke 1994, Aanen et al. 2002, Frøslev et al. 2003, Wei & Yao 2003). According to He (1985), *T. cylindricus* grows on nests of *Macrotermes barneyi* and *M. orthognathus.* In our field work in Xishuangbanna, Yunnan, *T. aurantiacus* was also found to be symbiotic with *Macrotermes* species. In Africa, *T. aurantiacus* was reported to be associated with the termite *Pseudacanthotermes militaris* (Batra & Batra 1979, Pegler & Vanhaecke 1994).

Termitomyces macrocarpus Z.F. Zhang & X.Y. Ruan in Acta Mycol. Sinica 5: 10 (1986). nom. invalid (Arts 37.1 and 37.2, ICBN).

Synonym of ***T. eurhizus*** (Berk.) R. Heim in Arch. Mus. Hist. Nat. Paris, Sér. 6, 18: 140 (1942).

In the protologue of *T. macrocarpus*, the specimens examined were listed as 'YUNNAN: Ad *Odontotermim formosanum* Shiraki et *O. yunnanensem* Tsai, alt. 1200–1900 m, Zhang Zheng-fu 1979–1981 (Typus Y B002, Y B003)'. Similar phrases were used in the description of the material in Chinese, but adding two locations as '禄劝、蒙自' (Luquan, Mengzi). Apparently, the description of the material is a mixture, with different locations, hosts and dates, and the designation of the type, either one specimen 'Y B002' or both specimens 'Y B002' and 'Y B003', is unclear. Although Ying & Zang (1994) was later able to specify 'YB002' from Luquan and 'YB003' from Mengzi and declared the type was deposited in the Herbarium of Yunnan Agricultural University (YB), the two numbered specimens are no longer traceable and the status of the type material cannot be further clarified. Because Zhang & Ruan (1986) did not unambiguously designate the holotype, the name '*Termitomyces macrocarpus* Z. F. Zhang & X. Y. Ruan' is invalid according to Arts 37.1 and 37.2 of ICBN (Greuter et al. 2000).

According to the description by Zhang & Ruan (1986), the basidiocarp of *T. macrocarpus* was large and robust, with pileus up to 38 cm diam., and the stipe base dark, often fuliginous-brown. In the illustration in the protologue, the badiocarps show typical characters of *T. eurhizus* in terms of the shape of pileus and stipe, and the pseudorhiza surface which is apparently darker than that of the stipe and possibly blackish. Because the pseudorhiza was not mentioned in their description, the dark stipe base described for *T. macrocarpus* by Zhang & Ruan (1986) is possibly referable to the pseudorhiza. *Termitomyces eurhizus* is recognized by its large fleshy, gray brown pileus with an obtusely rounded or broadly umbonate perforatorium, and the long, cartilaginous pseudorhiza encrusted with black-brown cuticle (Pegler 1977, Pegler & Vanhaecke 1994). The pileus of *T. eurhizus* can also extend to 36 cm in diam. (Pegler 1977), and the color of its pileus surface varies from almost white to dark brown. The morphological characters described by Zhang & Ruan (1986) and Ying & Zang (1994) for *T. macrocarpus* are identical with those of *T. eurhizus*. Therefore, *T. macrocarpus* is an additional synonym of *T. eurhizus*, although it was not validly published.

Discussion

Termitomyces is a paleotropical genus of macrofungi cultivated by termites belonging to the subfamily *Macrotermitinae* (*Isoptera*) (Heim 1942, 1977, Batra & Batra 1979, Bels & Pataragetvit 1982, Pegler & Vanhaecke 1994). In total, 68 taxa have been published in *Termitomyces*, with 81 names including combinations and autonyms. In recent decades, many new taxa were described from Asia, especially China (He 1985, Zhang & Ruan 1986, Wei et al. 2004) and India (Dhancholia et al. 1991, Natarajan 1975, 1977, 1979, Sathe & Daniel 1981, Sathe & Deshpande 1981). However, quite a few taxa described from this region have been found to be synonyms of early published names, i.e. taxa originally reported from India, *T. longiradicatus* Sathe & J. T. Daniel, *T. poonensis* Sathe & S. D. Deshp. and *T. quilonensis* Sathe & J. T. Daniel (Tang et al. 2006) and *T. indicus* Natarajan (Pegler & Vanhaecke 1994). Many more taxa of *Termitomyces* have been documented from Africa (Heim 1942, 1977, Otieno 1966, 1969, Pegler 1977, Van der Westhuizen & Eicker 1990, Mossebo et al. 2002) and the taxonomic status of some of them is also suspicious, i.e. *T. narobiensis* Otieno has been regarded as a synonym of *T. microcarpus* (Berk. & Broome) R. Heim, *T. biyii* Otieno and *T. rabuorii* Otieno considered a representative of an exannulate form of *T. letestui* (Pat.) R. Heim and of *T. mammiformis* R. Heim respectively, and *T. magoyensis* Otieno close to *T. schimperi* (Pat.) R. Heim (see Pegler 1977), and *T. umkowaanii* (Cooke & Massee) D. A. Reid very close to *T. eurhizus* (Pegler & Vanhaecke 1994). Mossebo et al. (2002) proposed eight new taxa based on material from Cameroon, but most of them resemble existing taxa. Careful revision of these taxa is required to confirm their taxonomic status.

Acknowledgements

The authors are grateful to Drs Peter Roberts and Zhu-Liang Yang for serving as pre-submission reviewers and for their valuable comments and suggestions, and to Prof. Fu-Sheng Huang for assistance in identification of the termite. This project is supported by a general grant (30470008) and the National Science Fund for Distinguished Young Scholars (30025002) from the National

Natural Science Foundation of China, the Key Research Direction of Innovation Programme (KSCX2-SW-101C) and the scheme of Introduction of Overseas Outstanding Talents, operated by the Chinese Academy of Sciences, and the National Hi-Tech Research and Development Plan (2004AA227100) from the Ministry of Science and Technology.

Literature Cited

Aanen DK, Eggleton P, Rouland-Lefèvre C, Frøslev TG, Rosendah S, Boomsma JJ. 2002. The evolution of fungus-growing termites and their mutualistic fungal symbionts. Proceedings of the National Academy of Sciences of the United States of America 99: 14887–14892.

Batra LR, Batra SWT. 1979. Termite-fungus mutualism. In Batra, L R, 1979. Insect-Fungus Symbiosis. Nutrition, Mutualism and Commensalis. New York: Allanheld, Osmun & Co. 117–163.

Bels PJ, Pataragetvit S. 1984. Edible Mushrooms in Thailand, Cultivated by Termites. in Zhang, S. T. & T. H. Quimio. 1984. Tropical Mushrooms, Biological Nature and Cultivation Methods (2nd. edn). Hong Kong, the Chinese University Press. 445–461.

Dhancholia S, Bhatt JC, Pant SK. 1991. Studies on some Himalayan agarics. Acta Botanica Indica 19: 104–109.

Frøslev TG, Aanen DK, Læssøe T, Rosendahl S. 2003. Phylogenetic relationships of *Termitomyces* and related taxa. Mycological Research 107: 1277–1286.

Greuter W, Mcneill J, Barrie FR, Burdet H-M, Demoulin V, Filgueiras TS, Nicolson DH, Silva PC, Skog JE, Trehane P, Turland NJ, Hawksworth DL. 2000. International Code of Botanical Nomenclature (St Louis Code). Koeltz Scientific Books, Königstein, Germany.

Guo D-Z. 1995. *Termitomyces* species on Nantai Island, Fuzhou. Edible Fungi 17: 5. (in Chinese).

He S-C. 1985. Taxonomic studies of *Termitomyces* from Guizhou Province of China. Acta Mycologica Sinica 4: 103–108.

Heim R. 1942. Nouvelles Études sur Les Agarics Termitophiles d'Afrique Tropicale. Archives du Muséum National d'Histoire Naturelle, Séries. 6, 18: 1–60, pls 9–12.

Heim R. 1977. *Termites et Champignons*. Société Nouvelle Des Éditions Boubée, Paris. 1–206

Hu Q-X, Cang R, Xiang H. 2000. Investigation of wild *Termitomyces* in Shuicheng County, Guizhou. Edible Fungi 22: 7. (in Chinese).

Huang N-L. 1998. Cloured Illustrations of Macrofungi (Mushroom) of China. Beijing: Chinese Agricultural Press. (in Chinese).

Kirk PM, Cannon PF, David JC, Stalpers JA. 2001. Dictionary of the Fungi. 9th edn. CAB International, Wallingford, UK.

Mossebo DC, Amougou A, Atangana RE. 2002. Contribution a l'étude du genre *Termitomyces* (*Basidiomycètes*) au Cameroon: écologie et systematique. Bulletin de la Société Mycologique de France 118: 195–249.

Natarajan K. 1975. South Indian *Agaricales* I. *Termitomyces*. Kavaka 3: 63–66.

Natarajan K. 1977. A new species of *Termitomyces* from India. Current Science 46: 679–680.

Natarajan K.1979. South Indian *Agaricales* V: *Termitomyces heimii*. Mycologia 71: 853–855.

Otieno NC. 1966 [as 1964]. Contributions to a knowledge of termite fungi in East Africa: the genus *Termitomyces* Heim. Proceedings of East African Academy 2: 108–120.

Otieno NC. 1969 [as 1968]. Further contributions to a knowledge of termite fungi in East Africa: the genus *Termitomyces* Heim. Sydowia 22: 160–165.

Pegler DN. 1977. A Preliminary Agaric Flora of East Africa. Kew Bulletin. Additional Series 6: 277–295.

Pegler DN, Vanhaecke M. 1994. *Termitomyces* of Southeast Asia. Kew Bulletin 49: 717–736.

Rouland-Lefevre C, Diouf MN, Brauman A, Neyra M. 2002. Phylogenetic relationships in *Termitomyces* of ITS: a first approach to elucidate the evolutionary history of the symbiosis between fungus-growing termites and their fungi. Molecular Phylogenetics & Evolution 22: 423–429.

Sathe AV, Daniel J. 1981 [as 1980]. Maharashtra Association for the Cultivation of Science (MACS), Monograph No. 1: *Agaricales* (Mushrooms) of south west India (Pune): 75–108.

Sathe AV, Deshpande S. 1981 [as 1980]. Maharashtra Association for the Cultivation of Science (MACS), Monograph No. 1; *Agaricales* (Mushrooms) of South West India (Pune): 9–42.

Shao L-P, Xiang C-T. 1997. Mushrooms in the forest of China. Harbin: Northeast Forestry University Press. (in Chinese).

Tang B-H, Wei T-Z, Yao Y-J. 2006 [as 2005]. Type revision of three *Termitomyces* species from India. Mycotaxon. 94: 93–102.

Van der Westhuizen GCA, Eicker A. 1990. Species of *Termitomyces* occurring in South Africa. Mycological Research 94: 923–937.

Wei T-Z, Yao Y-J. 2003. Literature review of *Termitomyces* species in China. Fungal Science 18: 39–54. (in Chinese).

Wei T-Z, Yao Y-J, Li T-H. 2003. First record of *Termitomyces entolomoides* in China. Mycotaxon 88: 433–438.

Wei T-Z, Yao Y-J, Wang B, Pegler DN. 2004. *Termitomyces bulborhizus* sp. nov. from China, with a key to allied species. Mycological Research 108: 1458–1462.

Wu X-L. 1990. Macrofungi in Guizhou Province. Guiyang: Guizhou People's Republic Press. (in Chinese).

Ying J-Z, Zang M. (eds.) 1994. Economic Macrofungi from Southwestern China. Beijing: Science Press. (in Chinese).

Zang M, Li B, Xi JX. 1996. Fungi of the Hengduan Mountains. Beijing: Science Press. (in Chinese).

Zhang X-Y. 1991. Illustration of Edible Fungi and Poisonous fungi in Guizhou. Guiyang: Guizhou Technological Publisher. (in Chinese).

Zhang Z-F, Ruan X-Y. 1986. A new species of *Termitomyces-Termitomyces macrocarpus* Zhang et Ruan sp. nov. Acta Mycologica Sinica 5: 10–13. (in Chinese).

MYCOTAXON

Volume 95, pp. 295–300 January–March 2006

Two new species of *Marasmius* (Basidiomycota, *Marasmiaceae*) from Brazil

Carla Puccinelli & Marina Capelari

puccinellic@yahoo.com.br
Instituto de Botânica, Seção de Micologia e Liquenologia
Caixa Postal 4005, 01061-970 São Paulo, SP, Brazil

Abstract—Two new species of *Marasmius*, *M. pseudosetosus* and *M. dimorphus*, collected in Atlantic Forest at São Paulo city, SP, Brazil are described, illustrated and discussed. Both species belong to *Marasmius* sect. *Sicci* subsect. *Siccini* series *Leonini*.

Key words—*Agaricales*, taxonomy, biodiversity

Introduction

In the course of making collections of *Marasmius* for the revision of this genus in the Parque Estadual das Fontes do Ipiranga (PEFI), São Paulo, SP, Brazil, two new species have been collected and are here described. Both species are classified in *Marasmius* sect. *Sicci* Singer, subsect. *Siccini* Singer, series *Leonini* Singer (Singer 1976). This group of *Marasmius* is characterized by the hymeniform pileal surface composed by smooth or *Siccus*-type broom cells without setae, absence of pleurocistidia and stipe glabrous without dermatocystidioid hairs or setae.

Until now, eight species of this group have been reported for PEFI by Grandi et al. (1984) and Pegler (1997): *M. berteroi* (Lév.) Murrill, *M. haediniformis* Singer, *M. leoninus* Berk., *M. phaeus* Berk. & M.A. Curtis, *M. pusio* Berk. & M.A. Curtis, *M. rhabarbarinus* Berk., *M. ruber* Singer and *M. subrotula* Murrill.

The microscopic analyses have been made from dried material rehydrated in 70% ethanol followed by 5% KOH and Melzer's reagent. The Qm represents the mean length/width quotient of the total spores measured. All the specimens are deposited in SP.

Taxonomic descriptions

Marasmius pseudosetosus C. Puccin. & Capelari **sp. nov.** **Figure 1**

Pileus 5-20 mm latus, campanulatus dein convexus, cum papilla centralis, margine levis, glaber, reticulatus, hygrophanus, pallide aurantiacus. Lamellae liberae sed confertae stipes, pseudocollarium in juvenis, albidae vel cremeae, acie concolorae, confertus, cum lamellulae. Stipes 30-42 × 1 mm, cylindricus, rubro-brunneus vel vinaceus, apice concolor lamellae, fistulosus, mycelium basium flavus. Basidiosporae 7.5-11.25 × 3.0-5.0 µm, ellipsoidae-fusoidae, leves, hyalinae, inamyloideae, tenuitunicatae. Pleurocystidia

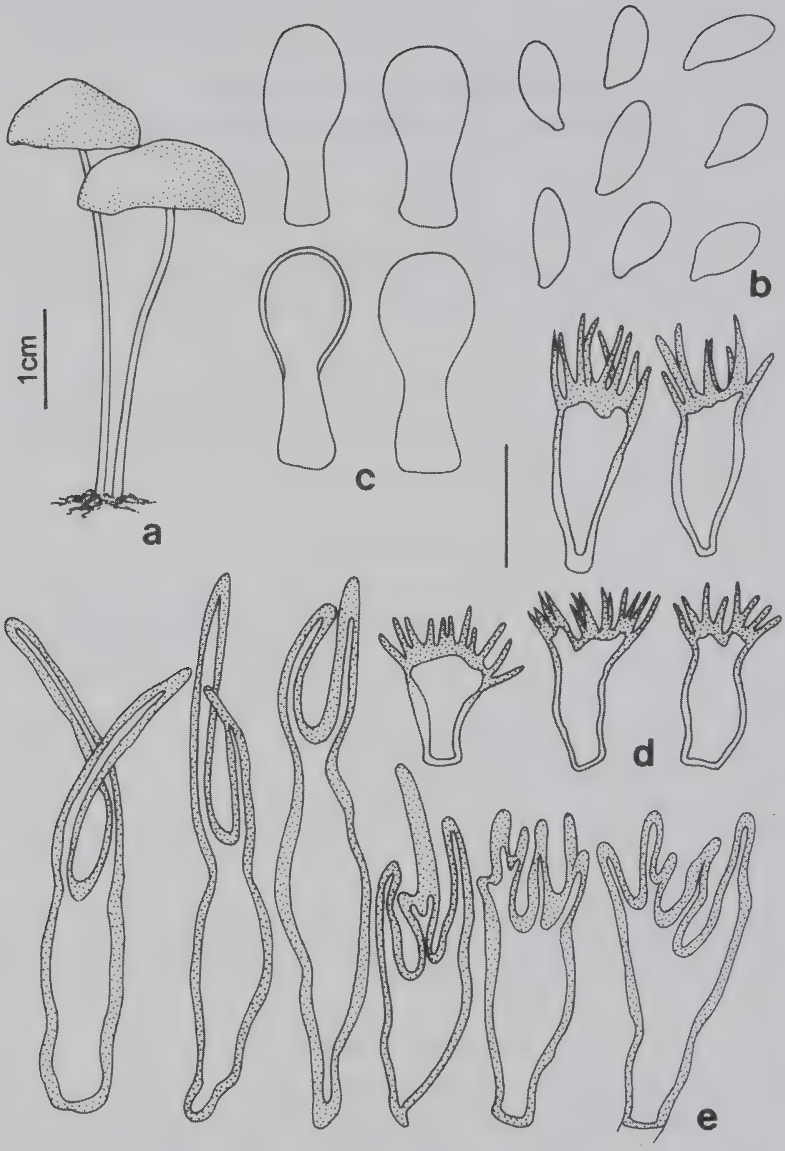


Figure 1. *Marasmius pseudosetosus* (holotype): a. basidioma, b. basidiospore, c. cheilocystidia, d. pileipellis cells, e. transitional cells. Scale bar = 10 μm.

nulla. Cheilocystidia similes cellulis hymenidermatis Marasmii sicci et clavatus pyriformis, 17.5-21.25× 7.5-8.75 µm hyalinus tenui vel crassitunicatis. Trama lamellarum regulare, ex hyphis dextrinoideis, 2.5-5 µm, tenuitunicatis, septatis, fibulae presentes. Pileipellis hymeniformis et cellulis similibus cellulis hymenidermatis Marasmii sicci et cellulis similibus setae. Cellulae setulosae 13.75-20 × 3.75-7.5 µm, clavate, hyalinae vel pallide luteo-brunneae, crassitunicatae, setulae ad apicem 6.25-8.75 µm; cellulae simili setae 31.25-55 × 5-8.75 µm, crassitunicatae, brunneae. Caulocystidia nulla. Dispersus ad folia sicca.

Holotypus: BRAZIL, SP, São Paulo. Parque Estadual das Fontes do Ipiranga, (23°39'S 46°37'W), 30 March 2005, Puccinelli 126 (holotypus in herbarium SP asservatur).

Pileus 5-20 mm diam., convex to campanulate, applanate when mature with a small central papilla, disc reticulate, margin smooth, glabrous, hygrophanous, pale orange and darker center. **Lamellae** free, but very close to the stipe, pseudocollarium present in the young specimens, with lamellulae, close, dirty white to cream, with concolorous edge. **Stipe** 30-42 x 1 mm, cylindrical, glabrous, central, hollow, reddish brown to vinaceous, becoming paler at apex where it is concolorous with the lamellae, yellowish cream, with a well-developed basal mycelium. **Context** thin, dextrinoid. **Basidiospores** 7.5-11.25 × 3.0-5.0 µm (Qm = 2.50, n = 40 basidiospores), ellipsoid-fusoid, smooth, hyaline, inamyloid, thin-walled. **Basidia** not observed. **Pleurocystidia** absent. **Cheilocystidia** of two types: a) similar to the broom cells from pileal surface, hyaline and very rarely found; b) predominantly clavate-pyriforme, 17.5-21.25 × 7.5-8.75 µm, smooth, hyaline, thin to thick-walled. **Pileipellis** hymeniform, composed of *Siccus*-type broom cells and transitional cells between broom cells and structures that resemble setae. *Siccus*-type broom cells with main body 13.75-20 × 3.75-7.5 µm, clavate, hyaline to brown or gold, thick-walled; apical setulae 6.25-8.75 µm; transitional cells 31.25-55 × 5-8.75 µm, scattered, brownish, thick-walled, some with similar shape to the broom cells. **Lamellar trama** regular, dextrinoid, with hyaline, thin-walled, septated hyphae, with clamp-connections, 2.5-5 µm diam. **Stipe** with cortical hyphae 3.75-7.5 µm diam, parallel, brownish yellow, dextrinoid, thick-walled; medullary hyphae 2.5-3.75 µm diam, hyaline, weakly dextrinoid, thin-walled. **Caulocystidia** absent. **Basidioma** scattered on dry leaves in the litter.

Material examined: BRAZIL, SP, São Paulo. Parque Estadual das Fontes do Ipiranga, (23°39'S 46°37'W), 30 March 2005, Puccinelli 125 (SP); Puccinelli 126 (holotype, SP); 01 April 2005, Puccinelli 131 (SP); 132 (SP).

Comments: This species is characterized by the hygrophanous, light orange, reticulated pileus, the close lamellae with lamellullae and stipe colour. Microscopically by the small and ellipsoid-fusoid basidiospores, absence of pleurocystidia, by the presence of dimorphic cheilocystidia (those similar with the broom cells are very difficult to find) and scattered transitional cells between setiform structures mixed with the broom cells in the pileipellis, (easily visible when the material is squashed). These structures are easily recognized by the larger size, the thickness of the wall and the deep colour. Among the species of *Marasmius* sect. *Sicci* with true setae in the pileipellis (series *Spinulosi* (Clémençon) Desjardin), *M. pseudosetosus* differs by the absence of setae in the pileipellis, by the pileus and stipe colour and by the dimorphic cheilocystidia. Macroscopically a close relative is *M. leoninus*, but they could be separated by the pileus colour and the peculiar pilleipellis structure of *M. pseudosetosus*.

Marasmius dimorphus C. Puccin. & Capelari **sp. nov.** **Figure 2**

> *Pileus 12–20 mm latus campanulatus dein convexus, margine crenata, glaber, sulcatus, spadiceus novus, brunneo–vinosus siccatus. Lamellae liberae, distantes, cremae, 17–18 lamella exclusus lamellulae. Stipes 4.8-5.6 mm, filiformibus, cavis, glabris, atrocastaneus, mycelium basium cremium. Basidiosporae 12.5–16.25 × 3.75-5 µm, clavate fusoideae, leves, hyalinae, inamyloideae, tenuitunicate. Pleurocystidia nulla. Cheilocystidia elementisque epicuticularibus setuligeris typi sicci sed minutus et hyalinus, crassitunicatae, 11.25–16.25 × 2.5-5 µm. Trama lamellarum regulare, hyphis hyalinis, dextrinoideis, 1.25–3.75 µm, tenuitunicatis, septatis, fibulae presentes. Pileipellis hymeniformis et cellulis similibus cellulis hymenidermatis Marasmii sicci, dimorphae. Minora cellulae setulosae 7.5–17.5 × 3.75–5 µm clavatae, crassitunicatae luteobrunneae et magna cellulae atrobrunnea, crassitunicatae, 32.5–45 × 5-6.25 µm. Caulocystidia nulla. Dispersus ad folia sicca.*
>
> *Holotypus: BRAZIL, SP, São Paulo. Parque Estadual das Fontes do Ipiranga, (23°39'S 46°37'W), 19 January 2005, Puccinelli 62 (holotypus in herbarium SP asservatur).*

Pileus 12-20 mm diam, campanulate to convex, margin crenate, surface glabrous but velutinous under a lens, sulcate, membranaceous, beige to light brown or pinkish brown, with darker center when fresh, turning vinaceous brown when dry. **Lamellae** free, distant (17-18), lamellulae absent, cream. **Stipe** 48-56 mm in length, central, glabrous, filiform, hollow, chestnut brown, with cream basal mycelium. **Context** thin, dirty white, dextrinoid. **Basidiospores** 12.5-16.25 × 3.75-5 µm (Qm = 3.8, n = 25 basidiospores), clavate-fusiform, smooth, hyaline, inamyloid, thin-walled. **Basidia** not observed. **Pleurocystidia** absent. **Cheilocystidia** like the broom cells of the pileipellis, but smaller and with pale color, slightly thick-walled, 11.25-16.25 × 2.5-5 µm. **Lamellar trama** regular; hyphae 1.25-3.75 µm diam., hyaline, dextrinoid, thin-walled, septated, with clamp-connections. **Pileipellis** hymeniform, composed of dimorphic *Siccus*-type broom-cells, normal broom-cells with main body 7.5-17.5 × 3.75-5.0 µm, clavate, light brown to light gold, thick-walled and larger broom-cells 32.5-45 × 5-6.25 µm, with few to many branches, deep brown, thick-walled. **Stipe** with cortical hyphae 6.25-8.75 µm diam, parallel, hyaline to brownish yellow, dextrinoid, thick-walled. **Caulocystidia** absent. **Basidioma** solitary on small sticks in litter.

> **Material examined**: BRAZIL, SP, São Paulo, Parque Estadual das Fontes do Ipiranga, (23°39'S 46°37'W), 19 January 2005, Puccinelli 62 (holotype SP), 07 April 2005, Puccinelli 142 (SP).

Comments: *Marasmius dimorphus* is characterized by the pileus colour, which ranges from beige to light brown, with darker center when fresh, some are pinkish brown and after drying they become vinaceous brown, with distant cream gills. The microscopical features of the broom cells of this species are very distinct, being dimorphic and differing in shape and size. The larger broom cells differ from the normal ones by the color, the thickness of the wall, the absence of a visible delimitation between the basal body and the setulae and by the few branches of the apical portion. They are also larger and more abundant near the centre and smaller and less present near the margin. These characteristics distinguish this interesting species from all other described species of *Marasmius*.

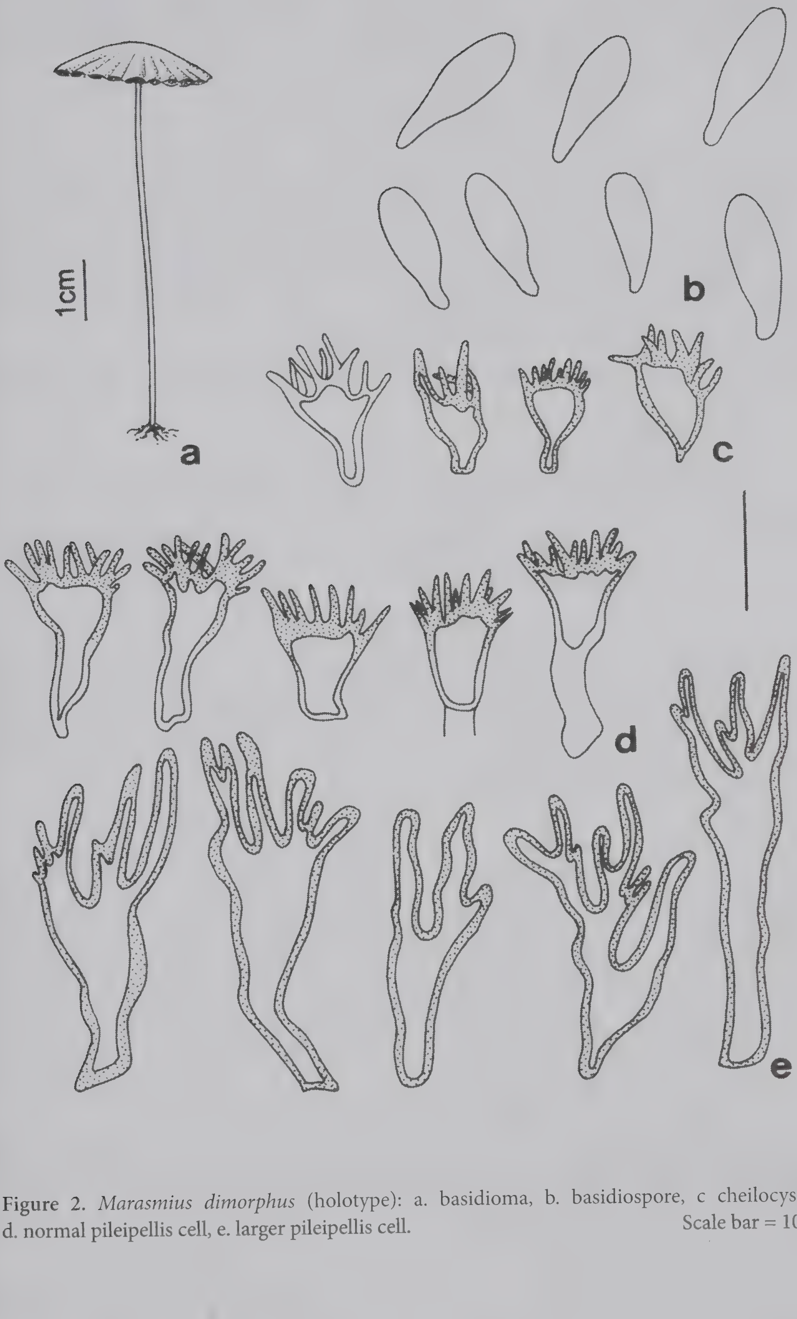


Figure 2. *Marasmius dimorphus* (holotype): a. basidioma, b. basidiospore, c cheilocystidia, d. normal pileipellis cell, e. larger pileipellis cell. Scale bar = 10 µm.

Acknowledgments

A special thanks to Maria Cecília Tomasi for inking the illustrations, Dr. Jefferson Prado, Instituto de Botânica, for revision of the Latin diagnosis. We also thank Dr. Vladimir Antonín, Moravian Museum, Dept. of Botany, and Dr. Dennis E. Desjardin, San Francisco State University, for their careful review of the manuscript. This study was supported by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP, grant 04/04319-2).

Literature cited

Desjardin DE, Horak E. 1997. *Marasmius* and *Gloiocephala* in the South Pacific Region: Papua New Guinea, New Caledonia and New Zealand taxa. Part 1: Papua New Guinea and New Caledonia taxa. Part 2: New Zealand taxa. In Petrini O, Petrini LE, Horak E. (eds.), Taxonomic monographs of Agaricales II. Bibliotheca Mycologica 168: 1-152.

Grandi RAP, Guzmán G, Bononi VL. 1984. Adições às Agaricales do Parque Estadual das Fontes do Ipiranga, São Paulo, SP, Brasil. Rickia 11: 27-33.

Pegler DN. 1997. The agarics of São Paulo, Brazil. Royal Botanic Gardens, Kew, 68 p.

Singer R. 1976. *Marasmiae* (Basidiomycetes, *Tricholomataceae*). Flora Neotropica. Monograph 17: 1-347.

MYCOTAXON

Volume 95, pp. 301–304 January–March 2006

Geastrum hirsutum: a new earthstar fungus with a hairy exoperidium

I. G. Baseia[1] & F. D. Calonge[2]

baseia@cb.ufrn.br
[1]Universidade Federal do Rio Grande do Norte, CB
Depto. Botânica, Ecologia e Zoologia, 59072-970, Natal, RN, Brazil

calonge@ma-rjb.csic.es
[2]Real Jardín Botánico, CSIC
Plaza de Murillo 2, 28014 Madrid, Spain

Abstract– *Geastrum hirsutum* was found growing on decaying wood on an abandoned termites nest in Atlantic rainforest. This rare species is easily recognized by its small and caespitose basidiome with a very hirsute exoperidium.

Key words– Gasteromycetes, *Phallales*, taxonomy, neotropics, Brazil.

Introduction

Historically, the *Geastrum* has been placed in the *Lycoperdales*. Recently, however, molecular data have prompted a great modification in the systematics of the gasteromycetes that has led to the transfer of this genus to the *Phallales* (Hibbett et al. 1997). Despite several previous publications (Rick 1961, Bononi et al. 1984, Kimbrough et al. 1995, Baseia & Milanez 2002, Baseia et al. 2003, Sobestiansky 2005), *Geastrum* is still not well known Brazil.

During the last decade, the extent of fungal diversity was widely discussed (Hawksworth 1993; Hyde 1997). Today we know that—in terms of species numbers—Kingdom Fungi is probably surpassed only by the insects (Hyde & Hawksworth 1997). It is estimated that there are at least 1.5 million fungal species in the world (Hawksworth 2001) but, up to now, only 72,000 species (or 5% of the projected total) have been described. In this context, our goal is to contribute to what is known about fungal diversity in Brazil and the world in general, with emphasis on the taxonomy and ecology of the gasteroid fungi.

Materials and methods

Fieldtrips have been carried out during May and June 2003, to the Parque Dois Irmãos (07°55' S and 34°52' W) and Reserva Ecológica do Gurjaú (08°14' S and 35°03' W). Both are remnants of the Atlantic rainforest located in the State of Pernambuco. Sporocarps were examined and photographed in the field. Macro and microscopic characters were determined according to Sunhede (1989). Colours were coded according to Kornerup & Wanscher (1978), with the indication "KW", bracketed in the text, and simultaneously described. They were dried up slowly and placed in containers with naphthalene.

Results

Geastrum hirsutum Baseia & Calonge, **sp. nov.** **Figs. 1-2**

> *Basidiomata juvene epigaeum, depresse globosum vel ovoideum, 4-8 mm latum, 5-10 mm altum, caespitosum, in subiculo pallide luteo. Exoperidium non hygroscopicum apertum 15-20 mm latum, fissum in 5-7 radios acutos, recurvos; stratum myceliale hirsutum, brunneoluteum; stratum medium pallide luteum, tenue; stratum internum brunneoluteum, tenue, persistens. Endoperidium sessile globosum cinereobrunneum laeve, 4-6 mm diam., cum; peristomio determinato, fibrilloso, cinereo. Gleba cinerea; columella indistincta; sporae globosae, 2.5-3 µm latae, brunneae, verrucis uniformibus subtilibus brevibus ornata. Hyphae capillitii longae, 1-1.5 µm latum, brunneae, verruculosae, non ramificatae.*
>
> **Holotypus:** *Brasil, Pernambuco, Recife, Reserva Ecológica do Gurjaú, ad lignum putridum, 12-VI-2003, leg. I. G. Baseia, UFRN-fungos 245.* **Paratypus:** *Dois Irmãos, 21/VI/2003, leg. I. G. Baseia URM 78711. MA-Fungi 67886.*

Basidiomata epigaeus when young, subglobose to obovate (Fig. 1), 4-8 mm broad, 5-10 mm high, caespitose, growing on a subiculum pale yellow (KW 4A3). Exoperidium, when open, 15-20 mm broad, split into 5-7 acute, recurving rays; nonhygroscopic (Fig. 1); mycelial layer densely hairy (Fig. 1), yellowish-brown (KW 5D8 to 5E7); hairs 1.5-3 mm long, made of hyphal aggregates with pointed ending, the hyphae are 2-5 µm diam., aseptate, clampless, with or without lumen, pale yellowish; medium layer pale yellowish (KW 4A3) to yellowish white (KW 4A2), thin; inner layer yellowish brown (KW 5E5), thin, persistent. Endoperidium smooth, brownish grey (KW 5D2), sessile, globose, 4-6 mm diam.; peristome definite, fibrillose, grey (KW 5B1). Gleba grey (KW 5F1), columella indistinct; spores globose, 2.5-3 µm broad, brown, with irregular short warts (Fig. 2a). Capillitium hyphae long, 1-1.5 µm broad, brown, covered with small wart-like outgrowths (Fig. 2b), not branching.

Discussion

Within *Geastrum* there are several species with some kind of hairy elements. Thus, *G. albonigrum* also has a hairy exoperidium (Calonge & Mata 2004), but it grows on soil, lacks any kind of a subiculum, and both basidiomata and spores are larger. On the other hand, *G. hieronymi* (Ponce de León 1968), *G. fimbriatum* var. *pseudohieronymi* (Calonge et al. 2005) and *G. setiferum* (Baseia & Milanez 2002) have a hairy or setose endoperidium, but they lack of any type of ornamentation on the exoperidium. Finally, *Phialastrum barbatum* (Sunhede 1989) shows a columella with a radiating sterile hyphal covering that resembles a beard.

An assembly of peculiar characteristics can easily identify this species: a mature developed subiculum and an exoperidium with a very hairy mycelial layer. Furthermore, it has very small spores 2.5-3 µm diam. The habitat is likewise unusual: decaying wood on an abandoned termites nest.

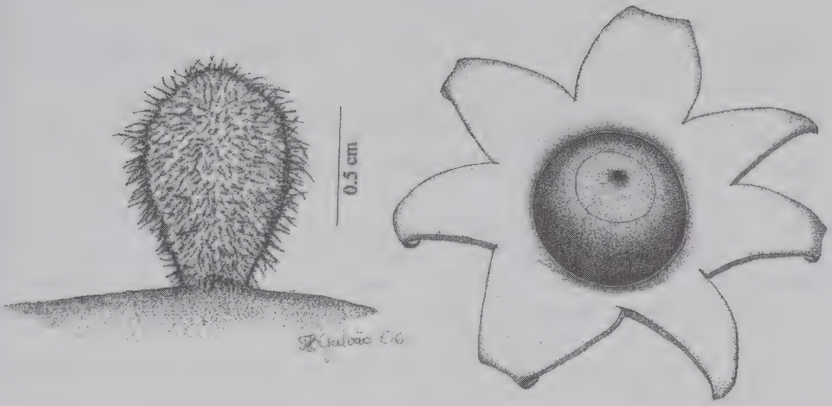


Fig. 1 ***Geastrum hirsutum*** (holotypus) **a**: Immature and mature basidiomata.

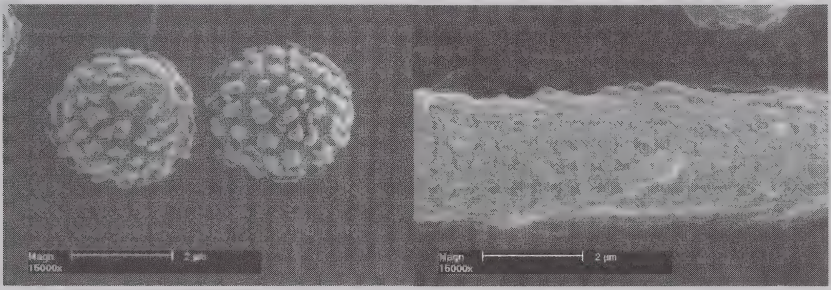


Fig. 2: ***Geastrum hirsutum*** (holotypus) **a**: Basidiospores under SEM, **b**: Capillitium surface.

Geastrum hirsutum is close to another tropical species, *G. schweinitzii* (= *G. mirabile*), in that both share several features in common: small, caespitose basidiomes growing on a well-developed subiculum that produce small spores and have a lignicolous habitat. In contrast, the exoperidium of *G. schweinitzii* has a smooth mycelial layer. All these features are enough to propose *G. hirsutum* as a new species.

Acknowledgments

We express our gratitude to the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for the partial financial support, and to Tereza Cristina de Oliveira Galvão for the illustrations. We also thank Professors H. Kreisel and G. Moreno for critical revision of the manuscript.

References

Baseia IG, Milanez AI. 2002. *Geastrum setiferum* (Gasteromycetes): a new species with a setose endoperidium. Mycotaxon 84: 135-139.

Baseia IG, Cavalcanti MA, Milanez AI. 2003. Additions to our knowledge of the genus *Geastrum (Phallales: Geastraceae)* in Brazil. Mycotaxon 85: 409-416.

Bononi VL, Guzmán G, Capelari M. 1984. Basidiomycetos do Parque Estadual da Ilha do Cardoso, V: Gasteromycetos. Rickia 11: 91-97.

Calonge FD, Mata M. 2004. A new species of *Geastrum* from Costa Rica and Mexico. Bol. Soc. Micol. Madrid 28: 331-335.

Calonge FD, Mata M, Carranza J. 2005. Contribución al catálogo de los Gasteromycetes (Basidiomycotina, Fungi) de Costa Rica. Anales Jardín Botánico Madrid 62 (1): 23-45.

Hawksworth DL. 1993. The tropical fungal biota: census, pertinence, prophylaxis and prognosis. In: Aspects of Tropical Mycology (eds. Issac S, Frankland JC, Watling R, Whalley AJS), Cambridge University Press, Cambridge: 265-293.

Hawksworth DL. 2001. The magnitude of fungal diversity: the 1.5 million species estimate revised. Mycological Research 105 (12): 1422-1432.

Hibbett DS, Pine EM, Langer E, Langer G, Donoghue MJ. 1997. Evolution of gilled mushrooms and puffballs inferred from ribosomal DNA sequences. Proceeding of the National Academy of Science USA 94: 12002-12006.

Hyde, KD. 1997. Can we rapidly measure fungal diversity? Mycologist 11(4): 176-178.

Hyde KD, Hawksworth DL. 1997. Measuring and monitoring the biodiversity of microfungi. In: Biodiversity of tropical microfungi (ed. Hyde, K. D.). Hong Kong University Press, Hong Kong: 141-156.

Kimbrough JW, Alves MH, Maia, LC. 1995. Basidiomycetes saprófitos presentes em troncos vivos e em folhedos de sombreiro (*Clitoria fairchiana* (Benth.) Howard). Biológica Brasílica 6 (1/2): 51-56.

Kornerup A, Wanscher JH. 1978. Methuen handbook of colours. Third edition. Eyre Methuen. London. 252 pp.

Miller OK, Miller HH. 1988. Gasteromycetes: Morphology and Developmental Features. Mad River, Eureka, CA, 157 pp.

Ponce de León P. 1968. A revision of the family *Geastraceae*. Fieldiana: Botany 31: 302-349.

Rick J. 1961. Basidiomycetes Eubasidii in Rio Grande do Sul. Iheringia, Ser. Bot. 9: 451-490.

Sobestiansky G. 2005. Contribution to a Macromycete Survey of the States of Rio Grande do Sul and Santa Catarina in Brazil. Brazilian Archives of Biology and Technology 48(3): 437-457.

Sunhede S. 1989. *Geastraceae* (Basidiomycotina). Morphology, ecology and systematics with special emphasis on the North European species. (*Synopsis Fungorum 1*). Fungiflora, Oslo, 535 pp.

MYCOTAXON

Volume 95, pp. 305–308 January–March 2006

Tricholoma lavendulophyllum, a new species from Yunnan, China

Fu-Qiang Yu[1,2], Yun Wang[3] & Pei-Gui Liu*

fqyu@mail.kib.ac.cn
[1]Kunming Institute of Botany, Chinese Academy of Sciences
Kunming 650204, China
[2]Graduate School of Chinese Academy of Sciences
Beijing 100039, China
[3]Shenyang Institute of Applied Ecology, Chinese Academy of Sciences
Shenyang 110016, China

Abstract—A new species of *Tricholoma* from the *matsutake* group, *Tricholoma lavendulophyllum,* is described and illustrated from Yunnan, China. It is characterized by its lavender lamellae and sweetish-aromatic smell resembling *T. matsutake.* The relationship of the new species to other closely related species is discussed.

Keywords—Matsutake, mushroom, taxonomy

Introduction

Tricholoma matsutake (S. Ito & S. Imai) Singer, commonly known as "Matsutake" or pine mushroom, is one of the most precious edible mushrooms in the world. There are a few closely related species, such as *T. bakamatsutake* Hongo, *T. caligatum* (Viv.) Ricken and *T. magnivelare* (Peck) Redhead, that are generally included in "Matsutake" (Wang et al., 1997). Although some taxonomical work has been done on this group (Zeller, 1934; Hotson, 1940; Hongo, 1960, 1974; Smith, 1979; Kytövuori, 1989), it is still insufficiently known, especially from China (Zang, 1990; Cao et al., 2003). In the fall of 2005, two collections of matsutake similar to *T. bakamatsutake* were obtained from Kunming wild edible mushroom markets. Careful examination of these two collections resulted in the description of a new species, *T. lavendulophyllum.*

Materials and Methods

Macroscopic characters were taken from fresh specimens. Descriptions of microscopic characters were observed under a Nikon E400 microscope with light and phase-contrast optics. Sections were made with a razor blade under the stereomicroscope, mounted in 5% KOH, and Melzer's solution, and then illustrated under the light microscope, with the aid of a drawing tube. Specimens examined were deposited in the Cryptogamic Herbarium of Kunming Institute of Botany, Academia Sinica (HKAS).

---

* Author for correspondence

Taxonomy

Tricholoma lavendulophyllum F.Q. Yu, **sp. nov.** **Fig. 1–4**

Pileus 45-70 mm latus, subumbonatus vel convexus, margine involuto dein recto; castaneo in centro, subcremeo ad marginem, fibrilloso. Lamellae lavandulus, adnatae vel subliberae. Stipes 80-95 × 12-15 mm, annulato, aequali vel basi incrassato, supra annulum albo, infra annulum pileo concolore, fibrilloso castaneo squamosoque. Carne alba, odor fragrans sicut in T. bakamatsutake. *Basidia clavata, 40-50 × 7.5-15 μm; basidiosporae (5.5) 6-7.5 × (4.5) 5-5.5 (6) μm, late ellipsoideae.*

Etymology—The specific epithet refers to the lavender-coloured lamellae

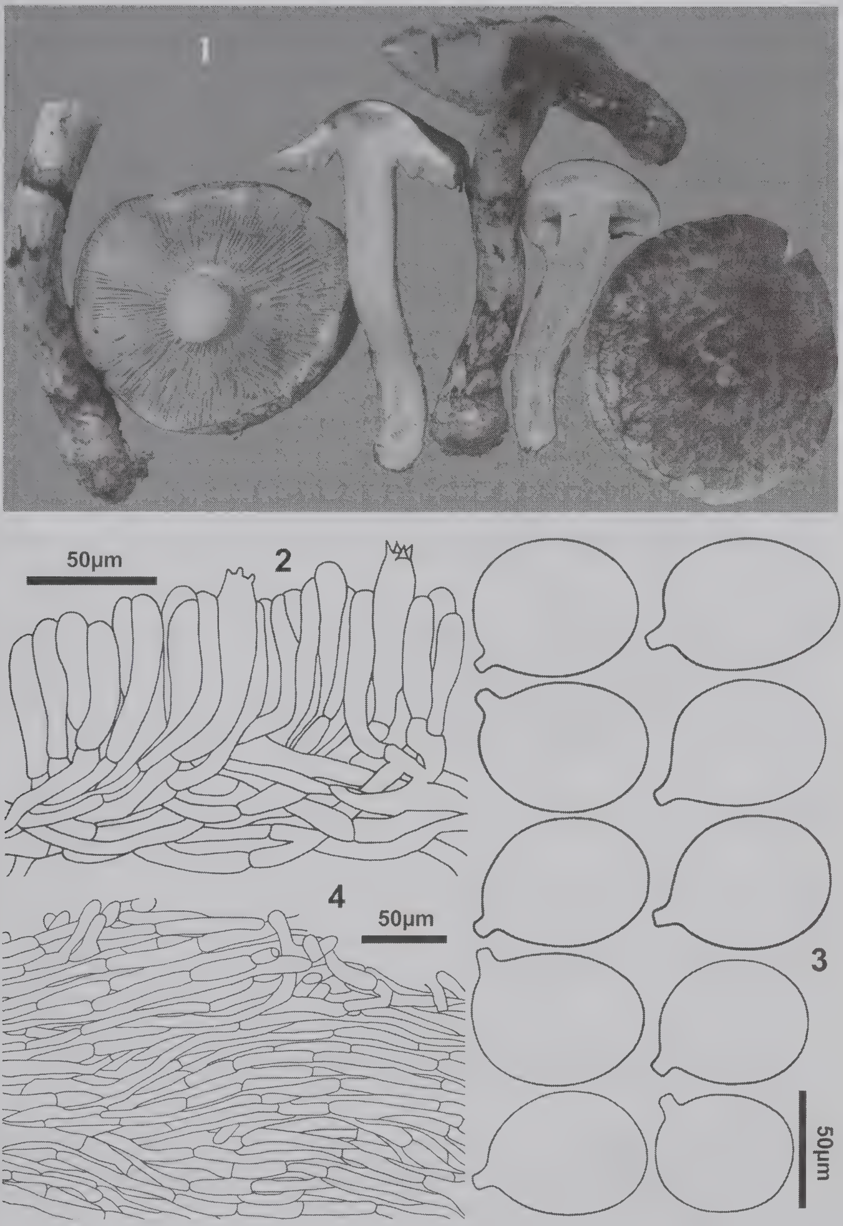
Pileus 45-70 mm broad, hemispherical to convex when young, becoming subumbonate to plano-convex when mature; surface slightly viscid when wet, chestnut brown to fuscous in the center, with appressed dark brown zoned scales, often becoming broken up into rather indistinct pieces; margin brown to whitish brown, inrolled when young. Context thick in the center, thin toward the margin, compact, white, taste sweetish. Aroma resembling *T. matsutake*, but stronger and with a slight smell of honey. Lamellae adnate, sinuate or arcuate, then separating from stem, pale to creamy lavender, with some lamellulae. Stipe 80-95 × 12-15 mm, equal or enlarged at the base, with an persistent but inconspicuous annulus on the upper part, 1.5-2.2 cm downward from the lamellae; concolorous with the pileus below the ring, with dark brown, appressed scales, whitish above the ring. Context white, solid, compact.

Pileipellis a layer of interwoven and thin-walled hyphae, 5-13 μm in diam., light yellowish brown, terminal elements subclavate to clavate, 7.5-9 × 22.5-67.5 μm. Lamellar trama subparallel, hyphae mostly 3-12 μm in diam., thin–walled, hyaline. Basidia 40-50 × 7.5-15 μm, clavate, hyaline, 4-spored, rarely 2- and 1-spored, sterigmata 2-5 μm long. Cystidia absent, but some clavate free hyphal ends present at the gill edge. Basidiospores (5.5-) 6-7.5 × (4.5-) 5-5.5 (-6) μm, Q= (1.18-) 1.25-1.33, broadly ellipsoid, thin-walled, hyaline, smooth, inamyloid. Stipitipellis with longitudinally arranged, appressed, parallel hyphae, 4-12 μm in diam., thin-walled, whitish brown to yellowish brown, terminal elements scattered, cylindrical, 6.8-9 × 30-54 μm, periclinally arranged.

Ecology: unknown.

Specimens examined—CHINA. YUNNAN PROVINCE: Kunming City, Aziying, wild edible mushroom market, 05 Aug. 2005, F. Q. Yu 1310B, HKAS49796; Kunming City, Wujing Road, wild edible mushroom market, 23 Jul. 2005, F. Q. Yu 1273, HKAS49804 (Holotype).

Notes--*T. lavendulophyllum* strongly resembles *T. bakamatsutake* in its appearance and aroma. It is sold as *T. bakamatsutake* at wild edible mushroom markets in Yunnan, China, but *T. lavendulophyllum* can be distinguished by its creamy lavender lamellae, and the absence of ventricose or flask-shaped cheilocystidia (Hongo, 1974). *T. fulvocastaneum* Hongo is another similar species to *T. lavendulophyllum* but differs in


1
50µm
2
4
50µm
3
50µm

Figs 1-4: *Tricholoma lavendulophyllum*, HKAS49804 (Holotype).
1. Basidiocarps; 2. Hymenium; 3. Basidiospores; 4. Section through pileipellis.

lacking of matsutake aroma, having whitish lamellae and a tapered base (Hongo, 1960). This species also resembles *T. caligatum* but the latter has whitish lamellae, relatively narrow spores, and a sweetish-bitterish to bitter taste (Kytövuori, 1989).

Acknowledgements

The authors wish to thank Dr. H. Knudsen of Copenhagen University, Dr. C. L. Ovrebo of University of Central Oklahoma, and Mr. E. Nagasawa of Tottori Mycological Institute for serving as pre-submission reviewers and for their valuable comments and suggestions. This project is partially supported by National Geographic Society of America (C56-04), Natural Science Foundation of Yunnan Province (2004C0050M), and National Natural Science Foundation of China (30470011).

Literature Cited

Cao ZM, Yao YJ, Pegler, DN. 2003. *Tricholoma zangii*, a new name for *T. quercicola* M. Zang (Basidiomycetes: *Tricholomataceae*). Mycotaxon 80: 159-164.

Hongo T. 1960. Notes on Japanese larger fungi (15). Journ. Jap. Bot. 35(3): 83-89.

Hongo T. 1974. Notes on Japanese larger fungi (21). Journ. Jap. Bot. 49(10): 294-304.

Hotson HH. 1940. The genus *Armillaria* in Western Washington. Mycologia 6: 776-790.

Kytövuori I. 1989. The *Tricholoma caligatum* group in Europe and North America. Karstenia 28: 65-77.

Smith AH. 1979. The Stirps *caligata* of *Armillaria* in North America. Sydowia Beih. 7: 368-377.

Wang Y, Hall IR, Evans LA. 1997. Ectomycorrhizal fungi with edible fruiting bodies I. *Tricholoma matsutake* and related fungi. Economic Botany 51: 311-327.

Zang M. 1990. A taxonomic and geographic study on the Songrong (matsutake) group and its allied species. Acta Mycologica Sinica 9(2): 113-127.

Zeller SM, Togashi K. 1934. The American and Japanese matsutake. Mycologia 26: 544-558.

MYCOTAXON

Volume 95, pp. 309–313 January–March 2006

Changes and additions to the North American lichen mycota – V

Kerry Knudsen

kk999@msn.com
Herbarium, Botany & Plant Sciences Department
University of California, Riverside
Riverside, CA, 92521-0124, USA

James C. Lendemer

lendemer@acnatsci.org
Lichen Herbarium, Department of Botany
The Academy of Natural Sciences of Philadelphia
1900 Benjamin Franklin Pky., Philadelphia, PA, 19103, USA

Abstract – *Lecanora utahensis is* placed in synonymy with *Lecanora peltasticoides*. A new combination is made: *Placopyrenium stanfordii. Dermatocarpon zahlbruckneri* (syn. *Catapyrenium zahlbruckneri*; *Placopyrenium zahlbruckneri*) is placed in synonymy with *P. stanfordii. Usnea cristatula* and *Cercidospora xanthoriae* reported new to North America.

1. ***Cercidospora xanthoriae*** (Wedd.) R. Sant., The Lichens and Lichenicolous Fungi of Sweden and Norway. p. 57. 1993.

Cercidiospora xanthoriae is known from Fennoscandia (Santesson 1993), Macaronesia (Hafellner 2002) and Europe (Sérusiaux et al. 1999). It is reported as new to North America, parasitic on *Xanthomendoza fallax* (Hepp ex Arnold) Søchting et al., in California. Javier Etayo verified the determination by the first author. The species usually has four strongly heteropolar spores per ascus.

USA. CALIFORNIA. SAN DIEGO CO.: Warner Hot Springs, Warner Hot Spring Ranch, 33°17'07" N, 116°37'31"W, elev. 1001 m., on *Xanthomendoza fallax* growing on *Quercus agrifolia, Knudsen* et al. *3900* (UCR, hb. Etayo).

2. ***Lecanora peltasticoides*** Hasse, The Bryologist, 17: 63. 1914. TYPE: USA, California, Riverside County, Palm Springs, on granite, 1901, *H.E. Hasse 861* (FH! holotype).

Syn. nov. *Lecanora utahensis* H. Magn., Acta Horti Gothob., 19(2): 39. 1952. TYPE: USA. Utah, Wayne County, Ekker's Ranch, on dry exposed sandstone, 6000 ft., *S. Flowers 359* (UPS, holotype; MIN! isotype).

Hasse (1914) published *Lecanora peltasticoides* based on a single collection made in 1901 in Palm Springs, California. The specific name refers to the similarity of the species to *Acarospora peltastica* Zahlbr., a synonym of *Acarospora strigata* (Nyl.) Jatta, which is common in the Palm Springs area.

Magnusson saw the holotype of *L. peltasticoides* in 1926 and annotated it as a *Lecanora*. By the fifties, he had long since forgotten the Hasse taxon and while studying the Utah lichen collections of the bryologist S. Flowers he re-described the species as *Lecanora utahensis* (Magnusson 1952). The holotype, according to Śliwa (Ryan et al 2004), is a poor fungus-infected specimen. But Śliwa expressed the opinion that southwestern collections represented the same species as Magnusson's holotype. The isotype of *L. utahensis* at MIN was given to Clifford Wetmore by Flowers and is a much better specimen than the holotype. Śliwa also reviewed the isotype and verified it as *L. utahensis*. The first author's examination of the isotype reveals it is conspecific with the holotype of *L. peltasticoides.*

During the work on the *Lecanora* treatment for the Sonoran flora project the holotype of *L. peltasticoides* was not seen by Śliwa. While on loan to ASU it was placed with the specimens for the *Acarospora* treatment based on William Weber's erroneous annotation of the specimen as "*Acarospora smaragdula* mod. *strigata oligospora*". Correcting the placement of the species in *Acarospora,* Knudsen (2003) recognized *L. peltasticoides* as a distinct species in need of further taxonomic study. The name thus appears in the most recent edition of the North American Checklist (Esslinger 2005).

Reexamination of the holotype of *L. peltasticoides* by the first author for Bjorn Owe-Larsson, to exclude the possibility that it represented a species of *Aspicilia*, revealed that it matched a collection of *L. utahensis* (*Knudsen 3448*, UCR!) from the San Bernardino Mountains of southern California. Subsequent examination of the isotype of *L. utahensis* revealed it to represent *L. peltasticoides*. Thus *L. utahensis* is here placed in synonymy with *L. peltasticoides.*

The thallus of *L. peltasticoides* is formed of chalky white areoles with immersed or sessile apothecia that are a red-brown. The ascospores are simple and hyaline, 13.3-16.3 x 5.9-7.4µm. For a full description by Śliwa see Ryan et al. (2004). The species has been collected on sandstone, granite, soil, and limestone, and occurs throughout western North America (Ryan et al. 2004), though it is infrequently collected. Growing in areas where *A. strigata* is common, *L. peltasticoides* is probably under-collected because of its superficial resemblance to the *Acarospora.*

3. ***Placopyrenium stanfordii*** (Herre) K. Knudsen**, comb. nov.**

Verrucaria stanfordii Herre, Proceedings of the Washington Academy of Sciences, 12: 42-43. 1910. TYPE: USA, California, on rocks in the foothills of Stanford University, one and a half miles back of Mayfield, 150 ft., *A.C.T.W. Herre 146* (F!, lectotype **designated here**).

Syn. nov. *Dermatocarpon zahlbruckneri* Hasse, Bryologist, 16:2. 1913. TYPE: USA, Los Angeles, on trap rock in Topanga Canyon, Santa Monica Range (FH, holotype)

Catapyrenium zahlbruckneri (Hasse) J.W. Thomson, Bryologist 90: 38. 1987.

Placopyrenium zahlbruckneri (Hasse) Breuss in Nash et al., Lichen Flora of the Greater Sonoran Region, 1: 396-397. 2002.

Herre (1910) published *Verrucaria stanfordii* based on a specimen collected in the Santa Cruz Mountains in Santa Cruz County in central California. After its description by Herre, *V. stanfordii* was forgotten. Hasse (1913) published the same species under the name *Dermatocarpon zahlbruckneri* three years later, perhaps unaware of Herre's specimens or their possible relevance with regard to his own lichen.

Later revisions of genera of Verrucariaceae placed in *Dermatocarpon* adopted Hasse's name for the taxon instead of Herre's name. In his revision of *Catapyrenium*, Thomson (1987) tranferred *D. zahlbruckneri* to *Catapyrenium*, based on the study of the holotype in the Farlow Herbarium (FH). When Breuss (2002) revised *Placopyrenium* in the Sonoran Desert region he transferred *C. zahlbruckneri* to *Placopyrenium.*

Recently, the first author (KK) reviewed the type of *V. stanfordii* and discovered it to be conspecific with *P. zahlbruckneri*. Agreeing with Breuss' circumscription of *Placopyrenium* from the related genera *Verrucaria* and *Catapyrenium*, but respecting the priority of Herre's original description, the new combination *Placopyrenium stanfordii* is made here. *Dermatocarpon zahlbruckneri* and its synonyms (*Catapyrenium zahlbruckneri* and *Placopyrenium zahlbruckneri*) are placed in synonymy with *P. stanfordii.*

For a description of *Verrucaria stanfordii* see Breuss (2002) under the name *Placyopyrenium zahlbruckneri*. The species is common in small populations mixed with other crustose lichens in southwestern North America. The specimen selected here as the lectotype was marked as a "co-type" but we have not been able to locate another specimen (and only one collection was cited in the protologue). Since the above annotation calls into question the status of the specimen as the holotype we have chosen to designate it as a lectotype to avoid ambiguity in the application of the name.

It should be noted that the epithet was originally published as "stanfordi". The epithet should be spelled "stanfordii". This orthographic error is corrected in the above new combination.

In his study of the lichen flora of Reno, Nevada, Herre (1911) reported *V. stanfordii.* Two specimens determined as *V. stanfordii* by Herre from Nevada were examined by the first author. The first collected at 5000 feet in Reno on March 5, 1910 (F!) and the second, collected in Marmol, Nevada, on April 30, 1910 (F!), are not *Placopyrenium stanfordii* but belong to the similar-looking *Verrucaria inficiens* group. The collections are characterized by subglose ascospores 10-13 x 7-9µm, evidence of being parasitic on two different lichen species (one a brown *Acarospora*), and as well as a broadly attached thallus (smaller than *P. stanfordii*). These collections correspond well with a new species of *Verrucaria* to be described by Breuss (in prep.) from the San Bernardino Mountains in southern California. Every specimen determined as *Verrucarria stanfordii* by Herre should thus not automatically be assigned to *Placopyrenium stanfordii.*

4. ***Usnea cristatula*** Motyka, Lich. Gen. *Usnea* Stud. Monogr. Pars Syst. 2(2): 643. 1938.
TYPE: Mexico, Michaocan, Morelia, Cerro Azul, elev. 3300 m, *A. Brouard* (LBL, holotype).

While revising A.W. Evans' collections of short apotheciate *Usnea* specimens for a revision the *Usnea strigosa* group (Lendemer & Ohmura in prep.) the second author enczountered specimens of a taxon unrelated to *U. strigosa* (Ach.) Eaton. Though superficially similar to *U. strigosa,* the thalli contained diffractaic acid (TLC!) and a pink medullary pigment like that of *U. ceratina* Ach. The specimens had been erroneously identified by Herre and Motyka (one each) as *U. strigosa*. Since both collections were from Texas, USA it seemed likely that the material represented a South/Central American taxon that reached the northern edge of its distribution in Texas. Examination of the available names led to the conclusion that the material represents *U. cristatula*, a species originally described from Michoacan, Mexico. The species is here reported for the first time from North America. The presence of diffractaic acid in the medulla as well as the medullary pigment easily separates this species from any other apotheciate taxon in North America and we hope that this report stimulates a search for modern collections from the region.

> USA. TEXAS. [without county]: without locality, *Lindheimer s.n.* (NY); Guadalupe River region, i.1931, *sine legit 76* (YU!). ATASCOSA CO.: low *Celtis*-live oak woods along Atascosa Creek, 7 miles north of Jourdanton along Texas 346, 12.vii.1963, *Pursell 5912* (NY). BEXAR CO.: mesquite woods, 18 miles east of San Antonio, 17.v.1940, *Hubricht B-1890* (F!, YU!).

Acknowledgments

We thank the following for helpful discussions, criticism and searching for specimens in other herbaria: Othmar Breuss, Robert Lücking, Clifford Wetmore, and Richard C. Harris. We also thank the curators of the following herbaria for loaning material cited herein: F, FH, MIN, and YU. We thank Javier Etayo for his verification of *Cercidospora xanthoriae,* Scott Eliason and Chris L. Wagner of the U.S. Forest Service for facilitating work in the San Bernardino Mountains, and Char Glacy and Nancy Nenow.

Literature Cited

Breuss, O. 2002. *Placopyrenium.* In: Nash III TH, Ryan BD, Gries C, and Bungartz F (eds.): Lichen Flora of the Greater Sonoran Desert Region. I. Lichens Unlimited, Arizona State University, Tempe, Arizona, pp. 393-397.

Breuss, O. In prep. *Verrucaria.* In: Lichen Flora of the Greater Sonoran Desert Region, vol. 3.

Esslinger, TL. 2005. A cumulative checklist for the lichen-forming, lichenicolous and allied fungi of the continental United States and Canada. North Dakota State University: http://www.ndsu.nodak.edu/instruct/esslinge/chcklst/chcklst7.htm. Fargo, North Dakota.

Halfellner, J. 2002. Bemerkenswerte Funde von Flechten und lichenicolen Pilzen auf makaronesischen Inseln VI. Über einige Neufunde. Fritschiana, 36: 11-17.

Hasse, HE. 1913. Additions to the lichen flora of southern California. VIII. Bryologist, 16: 1-2.

Hasse, HE. 1914. Additions to the lichen flora of southern California. No. 9. Bryologist, 17: 61-63.

Herre, AWCT. 1910. The lichen flora of the Santa Cruz Peninsula, California. Proceedings of the Washington Academy of Sciences, 12: 27-269.

Herre, AWCT. 1911. The desert lichens of Reno, Nevada. Botanical Gazette, 51: 286-297.

Knudsen, K. 2003. Type specimens: investigations and observations. Bulletin of the California Lichen Society, 10(2): 36-38.

Magnusson, AH. 1952. New crustaceous lichen species from North America. Acta Horti Gotoburgensis, 19(1): 31-49.

Ryan, BD., Lumbsch HT., Messuti MI., Printzen C., Śliwa L., and Nash III TH. 2004. *Lecanora.* In: Nash III TH, Ryan BD, Diederich P, Gries C, and Bungartz F (eds.): Lichen Flora of the Greater Sonoran Desert Region, Vol. 2. Lichens Unlimited, Arizona State University, Tempe, Arizona, pp. 176-286.

Santesson, R 1993. The Lichens and Lichenicolous Fungi of Sweden and Norway. SBT-förlaget, Lund. 240 pp.

Sérusiaux, E., Diederich, P., Brand, AM., and van den Boom, P. 1999. New or interesting lichens and lichenicolous fungi from Belgium and Luxembourg. VIII [Lichens et champignons nouveaux ou intéressants pour la flore de la Belgique et du G.-D. de Luxembourg. VIII]. Lejeunia, 162: 1-95.

Thomson, JW. 1987. The lichen genera *Catapyrenium* and *Placidiopsis* in North America. Bryologist, 90: 27-39.

MYCOTAXON

Volume 95, pp. 315–318 January–March 2006

Four new lichens from Turkey

Kenan Yazıcı*

kcagri_1997@yahoo.com
Karadeniz Technical University, Giresun Science and Art Faculty
Biology Department, Giresun, Turkey

Ali Aslan

aliaslan@atauni.edu.tr
Biology Department, Kazım Karabekir Education Faculty
Atatürk University, Erzurum, Turkey

Abstract—Four species of lichenized fungi, *Acrocordia cavata*, *Cladonia awasthiana*, *Parmelinopsis afrorevoluta* and *Usnea silesiaca*, are reported new to the lichen flora of Turkey. For each, a short description is presented.

Key words—Ascomycetes, biodiversity, Giresun, Gümüşhane, Trabzon, Zonguldak

Interest in the lichen flora of Turkey has greatly increased in recent years (Aslan 2000, Aslan et al. 2002a,b, John 1995, 1996a,b, John & Breuss 2004, John et al. 2000, Yazıcı & Aslan 2002, 2003, 2005, Yazıcı et al. 2004, 2005). This present paper is a further contribution to these works.

The present report is based on collections in the three different provinces of Giresun, Gümüşhane and Trabzon between 12 October 1999 and 10 August 2005. A stereo microscope, a light-microscope and the usual spot tests were used in the identification of the samples, together with the following references: Ahti & Upreti (2004), Clerc (1991, 1997), Halonen et al. (1988), Louwhoff & Elix (2002) Purvis at al. (1992). *Acrocordia cavata* has been stored in the herbarium of Kazım Karabekir Faculty of Education, Biology Department of Atatürk University, Erzurum and the others in the herbarium of Biology Department, Giresun Science and Art Faculty, Karadeniz Technical University.

Results

Acrocordia cavata (Ach.) R.C. Harris

Thallus crustose, immersed, grey-white, cortex absent. Photobiont *Trentepohlia*. Perithecia 0.2–0.7 mm diam., half-immersed, brown-black, involucrellum hemispherical, colourless-pale brownish exciple. Ostiole mostly eccentric and rarely papillate. Hamathecium of persistent, slender, sparingly branched or anastomosing, long-celled paraphysoids; no periphyses. Ascus 8–spored, cylindrical, fissitunicate; apical dome consiting of a broad ocular chamber surmounted by a hemispherical, meniscus-like structure, Ascospores uniseriate, colourless, elipsoid, the ends usually rounded, 1-septate, the median septum thick, 10–16 (–16.5) x 5.5–9.5 µm. Pycnidia absent.

This species normally grows on *Ilex* and *Corylus*. North America and Europe (Belgium, Germany, Sweden, Estonia, Norway, England, Netherlands, Luxemburg, France, Scotland, Slovenia, Spain).

> Giresun: Keşap–Karabulduk, Çamlıca village, on *Corylus avellana*, at 1100 m, 40° 50' 50" N, 38° 32' 30" E, 12 October 1999, Aslan 1270.

Cladonia awasthiana Ahti & Upreti

Primary thallus evanescent, consisting of very small (0.4–2.1 x 0.4 mm), crenate squamules bearing granules, sometimes squamules patches present from basal up to cups but mostly only basal portion of potedia. Podetia 35–45 mm tall, 2–2.5 mm across, 0.2–0.5 mm thick, grey to brownish, sometimes dichotomously branched, often proliferate from margins, axils mostly closed; surface ± corticated at the very base but otherwise ecorticate, consisting of granular soredia and microsquamules; soredia from basal up to cups. Podetial wall 220–270 µm, medulla 70–90 µm, surface of central canal minutely papillulate. Hymenial discs brown. Conidiomata mostly not common, terminal, clearly constricted at the base. K–, P+ red, UV+ white; fumarprotocetraric acid (major), homosekikaic acid (major), confumarprotocetraric and protocetraric acids (minor).

This species normally grows on bare, humous soil in the middle and upper Himalayan forested slopes, both in the Western and Eastern Himalayan Regions in India, at the elevations of 950–2800 m. Recorded before from the Indian states Arunachal Pradesh, Himachal Pradesh, Jammu & Kashmir, Sikkim, Iran and Uttaranchal (Ahti and Upreti 2004).

> Zonguldak: Çayköy, mainroad, on the mosses, 75 m, 41°22" N, 32°00" E, 10 August 2005, Yazici 1269.

Parmelinopsis afrorevoluta (Krog & Swinscow) Elix & Hale

Thallus grey, grey–white, some parts olive-green, loosely adnate, 4–12 cm wide. Lobes mostly abundant, subimbricate, sublinear, irregularly branched, to 3 mm wide; margins more or less entire or dentate, rarely irregularly incised, sometimes ± revolute, like slightly burned and wavy appearance, ciliate; cilia simple or partly forked, c. 1–1.2 mm long; cilia tips subrotund to subtruncate; granular soralia submarginal or in some parts laminally and pustular. Lower surface dark black and lobe margins mostly brown or rarely dark black, smooth to rugulose and with abundant rhizines; rhizines simple or bifurcate, to 2–2.5 mm long. Apothecia and pycnidia not seen. Medulla K–, P–, C+ rose, KC+ red; cortex C+ yellow; containing atranorin (minor), chloroatranorin (minor), gyrophoric acid (major), lecanoric acid (minor), 4, 5-Omethylhiascic acid (minor),5-O-methylhiascic acid (minor).

Grows in the warm-temperate and tropical regions of both hemispheres. Its distribution extends to Papua New Guinea, where it is uncommon, corticolous, occurring in disturbed and undisturbed montane forest at 1600-3600 m altitude. Known Australia, Ethiopia, Tanzania, Eastern North America and Sweden. (Elix 1994).

> Trabzon: Düzköy; Beypınar high plateau, on siliceous rock, at 700 m, 40° 48' N, 39° 20' E, 15 August 2004, Yazici 1273.

Usnea silesiaca Motyka

Thallus mostly pendulous (one of the specimens is subpendulous and partly shrubby), to 10 cm long (length of three samples are 6, 8 and 10 cm respectively), light–green or slightly yellow–green; cortex consisting of many annular cracks especially towards the base; base dark black, but black–brown base onwards to 2–3 cm.; cortex thick (10–20% of radius), K + red, C–, P–; medulla white, or sometimes slightly brown, compact, thin (9–15% radius), K+ red, P+ yellow, C–. Pseudocyphellae and soralia on cortex, soralia abundant, raised, confluent (but rare or absent on some branches, sometimes especially towards near the tips), mostly tuberculate, partly excavate and mostly larger than ½ of the branch diameter, K–, C–, P–. Isidia seen when young. Papillae generally indistinct. Branches are mostly isotomic and dichotomic, but anisotomic mostly near the tips. Thallus containing salazinic acid and accessory protocetraric and constictic acids.

Normally grows on conifers and deciduous trees, shrubs and hardwoods in open, humid forests at lower elevations. Common in humid coastal areas of North America (along the Pacific Northwest coast from Alaska and British Columbia south to California) and Europe (including Macaronesia, Austria, England, France, Netherlands Norway, Poland, Romania and Scotland) (Clerc 1991, 1997; Halonen et al. 1998)

> Gumushane: Kürtün; Çıkrıkdüzü high plateau, "Örümcek forests" on *Picea orientalis*, 1900m., 40° 35' 30" N, 39° 03'30" E, 02 June.2001, Yazici 1271.

Acknowledgements

We are grateful to Dr. André Aptroot and Dr. Javier Etayo for linguistic revision and helpful comments on an earlier draft of this paper and would like to thank Dr. André Aptroot for the identification and verification of *Acrocordia cavata,* Professor Teuvo Ahti for *Cladonia awasthiana,* Professor Jack A. Elix for *Parmelinopsis afrorevoluta* and Dr. Philippe Clerc for *Usnea silesiaca.*

References

Aslan A. 2000: Lichens from the Regions of Artvin, Erzurum and Kars (Turkey). Israel J. Plant Sci. 48: 143–155.

Aslan A, Yazıcı K, Karagöz Y. 2002a: Lichen Flora of the Murgul District (Artvin, Turkey) Israel J. Plant Sci. 50: 77–81.

Aslan A, Aptroot A, Yazıcı K. 2002b: New lichens from Turkey. Mycotaxon 84: 277–280.

Ahti T, Upreti DK. 2004: Two new species of *Cladonia* (*Lecanorales*) from the Himalayas. Biblioth. Lichenol. 88: 9–13.

Clerc P. 1991: *Usnea maderiensis* Mot. (ascomycète lichénisé): une espece meconnue de l'Europe et de l' Amérique du Nord. Candollea 46: 427–438.

Clerc P. 1997: Notes on the genus *Usnea* Dill. ex Adanson. Lichenologist 29: 209–215.

Elix JA. 1994: *Hypotrachyna.* Flora of Australia 55: 49–59.

Halonen P, Clerc P, Goward T, Brodo IM, Wulf K. 1988 : Synopsis of the Genus *Usnea* (Lichenized Ascomycetes) in British Columbia, Canada. The Bryologist 101(1): 36–40.

John V. 1995: Flechten der Turkei IV. Ergänzungen zum die Türkei betreffende lichenologische Schrifttum. Neunkirchen.

John V. 1996a. Preliminary catalogue of lichenized and lichenicolous fungi of Mediterranean Turkey. Bocconea 6: 173–216.

John V. 1996b. UTM–grid–mapping and distribution patterns of lichens in Turkey.– In Öztürk, M.A., Seçmen, Ö & Görk, G (eds.). Plant life in southwest and central Asia. Proceedings of 4th Plant life of Southwest Asia Symposium. 21–28 may 1995. İzmir: Ege Univ. Press. Bornova İzmir: 440–453.

John V, Seaward M.R.D, Beatty J.W. 2000. A neglected lichen collection from Turkey. Berkamsted School expedition 1971. Turk. J. Bot. 24: 239–248.

John V, Breuss O. 2004: Flechten der Östlichen Schwarzmeer-Region in der Türkei (Blam-Exkursion 1997). Herzogia 17: 137–156.

Louwhoff SHJJ, Elix JA. 2002: *Hypotrachyna* (*Parmeliaceae*) and allied genera in Papua New Guinea. Bibliotth. Lichenol. 81: 1–150.

Purvis OW, Coppins BJ, Hawksworth DL, James PW, Moore DM. 1992: The Lichen Flora of Great Britain and Ireland. Natural History Museum & British Lichen Society, London.

Yazıcı K, Aslan A. 2002: New records for the lichen flora of Turkey. Turk. J. Bot. 26: 117–118.

Yazıcı K, Aslan A. 2003: Lichens from the Regions of Gümüşhane, Erzincan and Bayburt (Turkey). Cryptogamie, Mycol. 24: 287–300.

Yazıcı K, Aslan A. 2005: Six new lichen records from Turkey. Mycotaxon 93: 359–363

Yazıcı K, Aslan A, Aptroot A. 2004: Four new lichen species from Turkey. Mycotaxon 90: 177–180.

Yazıcı K, Aslan A, Aptroot A. 2005: New lichen records from Turkey. Mycotaxon 92: 341–344.

MYCOTAXON

Volume 95, pp. 319–322 January–March 2006

Two new species in the genus *Morchella* (*Pezizales, Morchellaceae*) from China

Shu-Hong Li[1], Yong-Chang Zhao*[2],
Hong-Mei Chai[3] & Ming-Hui Zhong[4]

*[1]lmorelsh@yahoo.com.cn [2]yczhao@public.km.yn.cn**
[3]chm722@km169.net [4]yaasmushroom@yahoo.com.cn

Yunnan Key Lab of Agricultural Biotechnology
Kunming 650223, P. R. China

Abstract—Two new species of *Morchella, M. meiliensis* and *M. deqinensis,* collected from Yunnan, China are described and illustrated. The relationship of these two new species to other closely related species is discussed.

Key words— morel, ascocarp, taxonomy

Introduction

One thousand and thirty specimens of *Morchella* were collected from southwestern China and examined in the recent years. A total of ten species were identified, including two new species collected from Deqin County, Yunnan Province. Descriptions and illustrations of the two species, *M. meiliensis* and *M. deqinensis,* and comparisons with other morel species are provided below.

Taxonomy

Morchella deqinensis Shu H. Li, Y.C. Zhao, H.M. Chai & M.H. Zhong, **sp. nov.**

Fig. 1 a-f

> *Ascomata 5.2-9.5 cm alata, intus excavata. Capitulae 2.3–4.3 × 1.8–3.2 cm, ovoideae vel subconicae. Stipes conicus, cavus, 2.6-5.8 × 0.8-3.0 cm. Asci cylindricei, paraphysati, 8-spori, 9.91–10.49 × 105–148.65 μm. Sporidia elliposideo vel ovoideae, 6.4–8.1 × 9.2–9.6 μm.*
>
> **Etymology:** the specific epithet refers to the location of the holotype collected.

Ascomata 5.2–9.5 cm tall, hollow, with pileus completely attached to the stipe; pileus 2.3–4.3 × 1.8–3.2 cm, egg-shaped, sometimes broadly conical; ribs sparse and vertically arranged, dark greenish-brown; pits irregular quadrilateral, 2–3 times longer in length than in width, creamy or pale creamy, with many tiny crystals on the surface under stereomicroscope; context thin, firm, creamy or pale creamy; odor and taste not distinctive. **Stipe** 2.6–5.8 × 0.8–3.0 cm, hollow, conical, tapering, about 1/3–1/2 of the top, whitish when young, becoming dark rust when mature or dried, with white granules at the top and grooves at the base.

---

*Corresponding author

Hymenium 6–10 μm thick, hyaline, parenchyma, the bottom flattened; asci 9.91–10.49 × 105–148.65 μm, hyaline, cylindrical, circular on apex, 8-spored, single-space arranged, coming forth a circular orifice (8.5–9.0 μm in diameter) and a hemisphere cap on the apex and freeing ascospores from the orifice at maturity; ascospores 6.4–8.1 × 9.2–9.6 μm, hyaline, smooth, ellipsoid to ovoid, without oil droplets, thin-walled, spore print creamy; paraphyses 3.59–4.50 × 43.38–43.42 μm, hyaline, club-shaped, with a septum on the base.

> **Holotype:** CHINA, Yunnan, Deqin County, Xiaruo, alt.2800-3200 m, on the ground under coniferous or mixed forests, 20 April, 2005, Shu.H. Li (YKLAB, Yunnan Key Lab of Agricultural Biotechnology, Yunnan, China) YKLAB1002.

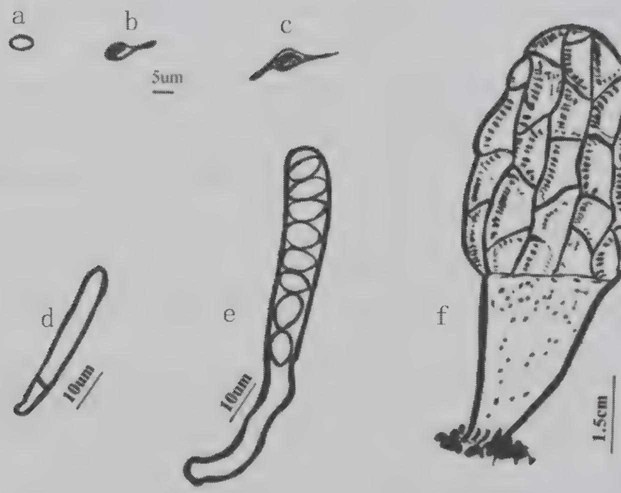


Fig.1—*Morchella deqinensis* (YKLAB1002). **a.** Ascospore. **b–c.** Germinating ascospores. **d.** Paraphysis. **e.** Ascus with ascospores. **f.** Fruiting body.

Morchella meiliensis Y.C. Zhao, Shu H. Li, H.M. Chai & M.H. Zhong, **sp. nov.**

Fig. 2 a-e

Ascomata 6.0–8.5 cm alata, intus excavata. Capitulae 3.5–4.8 × 1.2–2.0 cm, conicae. Stipes cylindricus, cavus, 2.3–3.5 × 0.8–2.0 cm. Asci cylindrcei, paraphysati, 8-spori, 5.18–5.87 × 91.16–94.44 µm. Sporidia elliposideo-oblonga, 4.67–5.10 × 5.20–5.66 µm.

Etymology: the specific epithet refers to the location of the holotype collected.

Ascomata 6.0–8.5 cm tall, hollow, completely attached to the stipe; pileus 3.5–4.8 × 1.2–2.0 cm, conical; ribs sparse and vertically arranged, dark brow to black; the hymenium of between the ridges merulioid; pits irregular-quadrilateral or rectangle, creamy to yellowish; context thin, firm when drying; odor and taste not distinctive. **Stipe** 2.3–3.5 × 0.8–2.0cm, whitish when fresh becoming waxy yellow when dry, cylindrical, with white granules at the top and grooves at the base.

Hymenium 8–15 µm, hyaline or pale, parenchyma, the bottom slightly wavy; asci 5.18–5.87 × 91.16–94.44 µm, hyaline, cylindrical, circular on the apex, 8-spored, single-space arranged; ascospores 4.67–5.10 × 5.20–5.66 µm, hyaline, smooth, ellipsoid, with oil droplets, thin-walled, swollen and darkening when germinating; spore print creamy; paraphyses 4.19–5.20 × 40.09–65.01 µm, dark, club-shaped.

Holotype: CHINA, Yunnan, Deqin County, Meili Snow Mountain, alt.2800-3500m, on the ground under broadleaf forests or mixed forests, 13 May, 2005, Y.C. Zhao (YKLAB, Yunnan Key Lab of Agricultural Biotechnology, Yunnan, China) YKLAB1080.

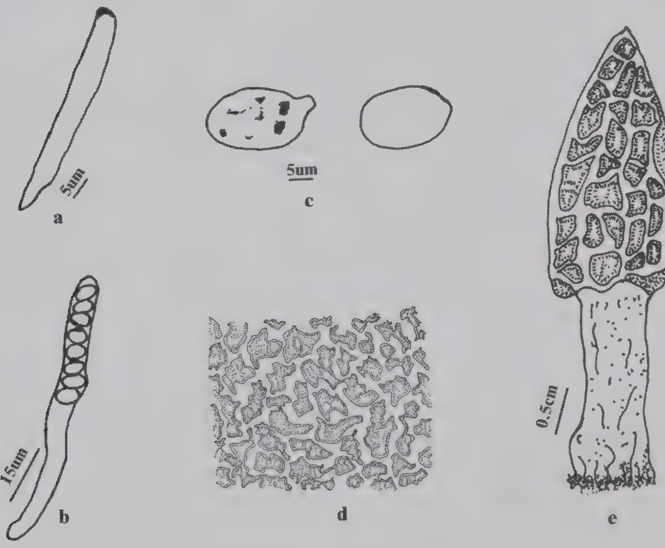


Fig.2—*Morchella meiliensis* (YKLAB1080). **a.** Paraphyses. **b.** Ascus. **c.** Germinating ascospores. **d.** Pores (under stereo-microscope). **e.** Fruiting body.

Discussion

M. deqinensis resembles *M. umbrina* Boud. (Huang 1998, Mao 2000) but differs in its tapering stipe with white granules and grooves at the base. Also *M. umbrina* has smaller pits and a deeper color than *M. deqinensis.*

M. meiliensis is closely related to *M. angusticeps* Peck (Bi 1994, Zang 1996, Mao 2000, Kuo 2005) and *M. conica* Pers. (Zang 1996, Huang 1998, Mao 2000). The merulioid hymenium, lighter colored ribs, and club-shaped paraphyses distinguish *M. meiliensis* from the other two species.

Acknowledgements

We would like to thank Profs. Yun Wang (Crop & Food Research, New Zealand) and Xin-Chang Luo (Department of Plant Protection, Huazhong Agricultural University) for paper review and good advice in English writing. This study was supported in part by Opening Grant of Shanghai Key Lab of Agricultural Genetics and Breeding (Shagb2005-02).

Literature Cited

Bi ZS, Zheng GY, Li TH. 1994. Macrofungi in Guangdong. Guangdong Science & Technology Press, Guangzhou.

Chen JY, Liu PG. 2005. A new species of *Morchella* (*Pezizales,* Ascomycota) from southwestern China. Mycotaxon 93: 89-93.

Huang NL. 1998. Colored Illustrations of Macrofungi of China. China Agricultural Press, Beijing.

Kuo M. 2005. The black morels. Retrieved from the MushroomExpert.Com web site: http://www.mushroomexpert.com/morchella_angusticeps.html

Kuo M. 2005. The *Morchellaceae:* True morels and verpas. Retrieved from the Mushroom Expert. Com Web site: http://www. mushroomexpert.com/morchellaceae.html

Mao XL. 2000. The Macrofungi in China. Henan Science and Technology, Zhengzhou.

Zang M. 1996. Fungi of the Hengduan Mountains. Science Press, Beijing.

MYCOTAXON

Volume 95, pp. 323–330 January–March 2006

Notes on some species of the lichen genus *Lecidea* from India

D.K. Upreti[1]*, S. Nayaka[1]
& M.P. Andreev[2]

*upretidk@rediffmail.com
[1]Lichenology Laboratory, National Botanical Research Institute, Rana Pratap Marg
Lucknow – 226 001, India

[2]Komarov Botanical Institute of the Russian Academy of Sciences
2, Prof. Popov St., 197376, St. Petersburg, Russia

Abstract—A detailed morphotaxonomic account of seven species of *Lecidea* from India is provided. *Lecidea confluens*, *L. plana*, and *L. tessellata* are new records for the Indian lichen mycota.

Key words—*Lecideaceae*, lichen flora

Introduction

The lichen genus *Lecidea* is characterized by crustose, heteromerous, continuous, areolate or verruculose or squamulose thallus lacking rhizines and decorticated or with a thin cortex and sometimes servediate, with photobionts being members of the Trebouxiophyceae. Apothecia are orbicular or angular, contorted, immersed, sessile or very shortly stalked, proper margin colourless, coloured or blackened, or closely woven hyphae; excipulum colourless or reddish or grayish; hypothecium colourless or coloured or ± black; paraphyses simple or branched with swollen, with free or conglutinate, capitate apices, free or conglutinate, *Lecidea*-type asci clavate, 8-spored; ascospores simple colourless, oblong-oval, ellipsoid, with thin wall, pycnidia immersed, with short conidia.

Awasthi (2000) listed 42 species of *Lecidea* s. lat. from Indian subcontinent, out of which about 20 species are known from the Karakorum and Khumbu Himal area of Nepal, five from Ceylon (Sri Lanka), and 17 species from India. Most of the taxa earlier listed under *Lecidea* from India are at present transferred to different lichen genera. The material of *Lecidea advena* Nyl. ex Hue, *L. albicans* Nyl., *L. caliginosa* Stirt., *L. fuscorubida* Nyl., *L. secernens* H. Magn., which were not traceable in the Indian herbaria, therefore are not included in the present study. *Lecidea nagalandica* G.P. Sinha & Kr.P. Singh, which Sinha & Singh (1987) described as a new foliicolous lichen from India, probably does not belong to *Lecidea* due to the presence of brown-black exciple.

The *Lecidea* species in India are widespread in higher altitudes (2000-4700 m) of the Himalayan region. The states of Uttaranchal and Himachal Pradesh in the western Himalayas include five each of taxa reported in the present study while only three species are found in Jammu and Kashmir.

Awasthi (1991) did not treat *Lecidea* species in his key to microlichens from the Indian subcontinent. The present communication provides a detailed morphotaxonomic account of seven species of *Lecidea* occurring in India according to the modern concept.

Materials and methods

Specimens were studied from the personal lichen collections of Dr. D.D. Awasthi (AWAS), Botany Department, Lucknow University Herbarium (LWU), and National Botanical Research Institute (LWG) preserved at LWG and representing all the phytogeographical regions of India. The specimens were examined morphologically, anatomically and chemically. For chemical spot reaction the usual reagents 5% potassium hydroxide (K), paraphenylenediamine (PD) and calcium hypochlorite (C) were applied.

Thallus and apothecial sections were cut using a microtome (Fuji-Japan) at 12-15 μm thickness and stained with lactophenol cotton blue. The chemical constituents were identified in solvent A (180 ml toluene : 60 ml 1-4-dioxane : 8 ml acetic acid) using the thin layer chromatography (TLC) technique by Culberson (1972) and Walker & James (1980).

Key to the *Lecidea* species from India

1a. Thallus brown to dark brown or yellow brown (medulla I+ violet) .... ***L. atrobrunnea***
1b. Thallus whitish, greyish to pale yellowish brown or grey brown **2**

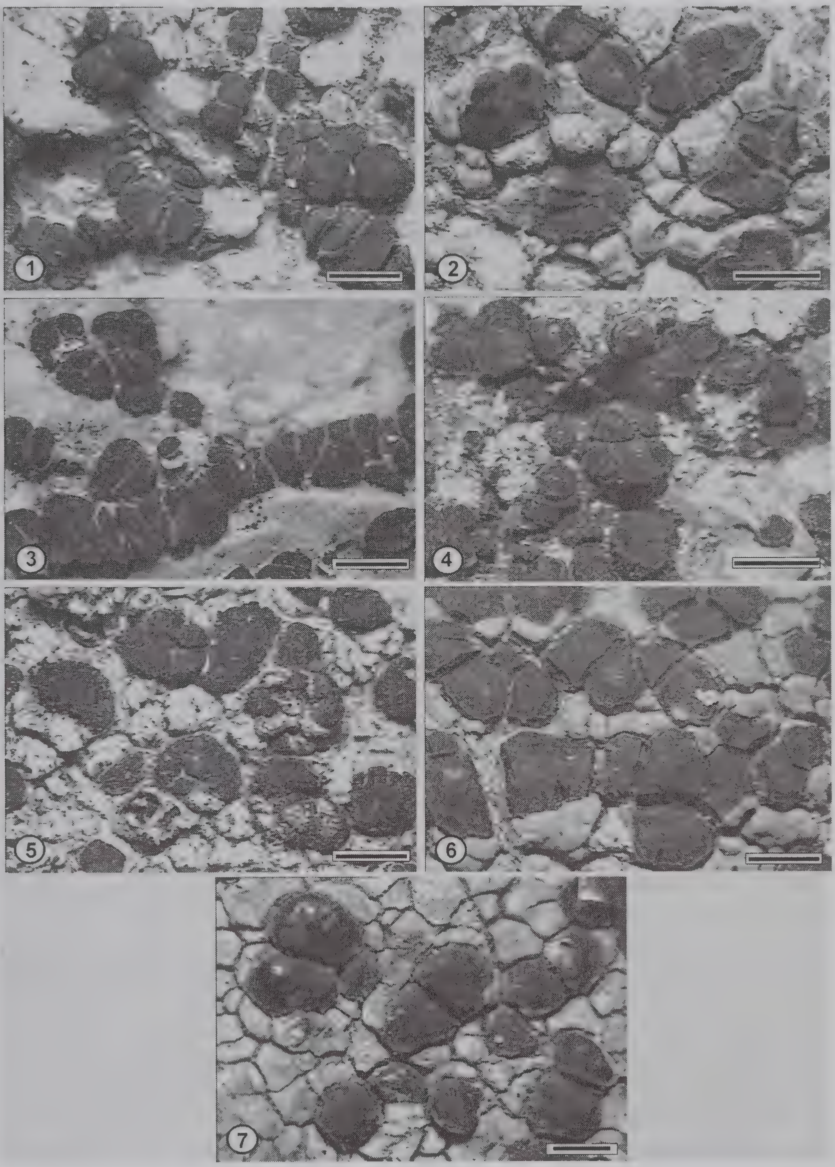
2a. Medulla I+ deep violet (thallus thick, grey, hypothecium brown to dark brown) .... ***L. confluens***
2b. Medulla I– 3

3a. Thallus K+ yellow (PD–, hypothecium pale to dark brown) .... ***L. lapicida***
3b. Thallus K– 4

4a. Hypothecium colourless, epihymenium deep green .... ***L. plana***
4b. Hypothecium brown to dark brown 5

5a. Apothecia immersed, thallus chalky white to grey, hypothecium pale brown .... ***L. tessellata***
5b. Apothecia not immersed **6**

6a. Excipulum usually broad, developed below the apothecium .... ***L. auriculata***
6b. Excipulum not developed below the apothecium .... ***L. paratropoides***



Figures–1-7. Habit

1. *Lecidea atrobrunnea*; **2.** *L. auriculata*;
3. *L. confluens*; **4.** *L. lapicida*;
5. *L. paratropoides*; **6.** *L. plana*;
7. *L. tessellata*.

Taxonomic descriptions

Lecidea atrobrunnea (Lam. & DC.) Schaer. Lich. Helv. Spic., sect. 3: 134 (1818). **Fig. 1**
– *Rhizocarpon atrobrunneum* Ramond ex Lam. & DC., Fl. Franç. ed. 3, 2: 367 (1805).

Thallus reddish brown with epinecral layer, squamulose-areolate, areolae rounded, plane, with whitish margin; hypothallus distinct, black; apothecia subimmersed to adnate, slightly constricted at base, upto 1.5 mm diam.; disc plane or somewhat convex in mature ones, epruinose, margin prominent, sometimes flexuose; excipulum 40–60 µm thick, blackish green externally, colourless internally; epithecium deep green to blackish green; hymenium 40–60 µm high, colourless, I+ blue; subhymenium 60–70 µm high, colourless, I+ blue; hypothecium brown, 80-100 µm high, I+ blue; paraphyses simple or sometimes branched, thickened at apices. Asci clavate, 40–50 x 10–13 µm; ascospores 8–10 x 4–6 µm; with obtuse ends.

Thallus K–, KC–, C–, PD–; a pale yellow spot at Rf class 5 in TLC.

> SPECIMENS EXAMINED–HIMACHAL PRADESH: Kangra district, Bir Tea Estate area, Billing Hills, 2000-2500 m, on exposed rocks, Upreti 213602/A (LWG); Lahul Spiti district, Baralacha La Pass, 4700 m, on exposed rocks, Upreti and Chatterjee 03-001776 (LWG); Shimla district, Narkanada, Hatu Peak, 3360 m, on rocks, Nayaka and Srivastava 02-67181 (LWG).

Lecidea atrobrunnea is distinguished by a deep brown thallus with an epinecral layer and a well developed hypothecial medulla, small ascospores, and I+ blue medulla.

The species is widely distributed in alpine regions of all the continents. Hertel (1977) reported the occurrence of this species from Reti Runi area in Spiti valley. In India the species is known to occur in alpine regions of Himalayas in Himachal Pradesh between altitudes of 2000–4700 m in Lahul Spiti, Kangra and Shimla districts.

Lecidea auriculata Th. Fr. Nova Acta Reg. Soc. Sci. Upsal., Ser. 3, 3: 213 (1860). **Fig. 2**

Thallus endolithic, or cracked-areolate, medulla I+ blue; hypothallus indistinct; apothecia black, sessile, constricted at base, 1.0–2.0 mm in diameter; disc plane, epruinose or sometimes slightly pruinose; margin distinct, sometimes flexuose; excipulum developed below the apothecium, 100–150 µm thick, dark brown externally, internal part light brown, hyphae intricate-radiating, 3–5 µm, thin walled; epithecium blue-green; hymenium colourless, 30–40 µm high; subhymenium 40–50 µm high, light brown; hypothecium light brown; subhypothecial medulla prominent and raised up, I+ blue; paraphyses simple, thickened at the apices. Asci clavate, 25–40 x 7–9 µm; ascospores ellipsoid with obtuse ends, 6–11 x 2.5–4 µm.

Thallus K–, KC–, C–, PD–; no lichen substances detected with TLC.

> SPECIMENS EXAMINED–JAMMU AND KASHMIR: Leh district, Ganglas area, 4000 m, on exposed rocks, Upreti and Chatterjee 03-001814/A (LWG). HIMACHAL PRADESH: Lahul Spiti district, Pasio, 3800 m, on exposed rocks, Upreti and Chatterjee 03-001742/A (LWG); Zinzibar, 4200 m, on exposed rocks, Upreti and Chattrjee 03-001756/A (LWG); Baralacha Pass, 4700 m, on exposed rocks, Upreti and Chatterjee 03-001772, 03-001774 (LWG).

The species is characterized by a well-developed excipulum below the apothecium, blue green epithecium, violet brown subhymenium, very narrow ascospores and the presence of confluentic acid.

According to Hertel (1977) this taxon has a bipolar distribution and is widespread and common in Arctic-alpine belt of the more humid high mountain rages in Europe, Asia and North America. In India the species is known to occur in alpine regions of Jammu and Kashmir and Himachal Pradesh between altitudes of 3800–4700 m. Awasthi (1963) first reported this species from Kangra district in Himachal Pradesh.

Lecidea confluens (Weber) Ach. Methodus Lich. 14 (1803). **Fig. 3**
– *Lichen confluens* Weber, Spicil. Fl. Goett. 180, tab II (1778).

Thallus cracked areolate, bluish grey to grey, medulla, I+ blue; prothallus black; apothecia adnate 0.5–1.2 mm diam., immersed to ± sessile, arising between the areoles, black; disc plane to slightly concave, epruinose; margin thick, prominent; epithecium green to olive green; hymenium colourless 70–100 μm high; hypothecium dark red brown to dark brown, 90–100 μm high; excipulum blackish at outer edge, ± colourless within. Asci clavate, 50–60 x 10–16 μm; ascospores ellipsoid, 10–12 x 4–6 μm.

Thallus medulla K–, KC–, C–, PD–; confluentic acid in TLC.

Specimens examined–JAMMU AND KASHMIR, Glumarg, at Khilanmarg, 2760 m, on boulders, Dange 77-535 (LWU-LWG). **UTTARANCHAL:** Chamoli district, Badrinath, south of temple near Brahmini village, 3100–3200 m, on boulders, Dange 76-740 (LWU-LWG), north of temple, way to Mana Village 3100–3250 m, on boulders, Dange 76-892 (LWU-LWG).

The species is know to occur on siliceous rocks in northern Eurasia and North America and is a new record for Indian lichen flora.

This species is similar to *L. lapicida* and *L. tessellata. L. confluens* prefers to grow in more humid habitats, has wider areoles, dark red brown to dark brown hypothecium, K+ yellow excipulum and smaller ascospores than *L. lapicida.* From *L. tessellata* it differs in having an almost colourless hypothecium.

Lecidea lapicida (Ach.) Ach. Method. Lich. 37, (1803). **Fig. 4**
– *Lichen lapicida* Ach., Lich. Suec. Prodrom. 61, (1798).

Thallus cracked areolate, whitish grey, changed brownish in herbarium, medulla I+ blue; prothallus black; apothecia appressed to adnate, 0.5–1.5 mm diam., immersed to ± sessile, arising between the areoles, black; margin thick prominent; disc plane or slightly concave; epruinose with dark green at outer edge; epihymenium greenish black, K+ blue green; hymenium colourless, 90–120 μm high; hypothecium pale to dark brown, 80–100 μm high; excipulum blue–green externally, pale brown internally, K+ yellow; Asci clavate, 40–60 x 10–16 μm; ascospores ellipsoid, 19–11 x 4–6 μm.

Thallus medulla K+ yellow, C–, KC–, PD+ yellow; stictic and constictic acid in TLC.

Specimens examined–UTTARANCHAL: Dehra Dun district, Mussoorie hills, way to Lal Tibba, 2250 m, on rocks, Joshi 75-340 (LWU-LWG). **HIMACHAL PRADESH:** Lahul Spiti district, 10 km before Sarchu, 4000 m, on rocks, Upreti 03-001783 (LWG).

The species is widespread in northern and southern hemispheres on siliceous rocks. Earlier, Hertel (1977) reported this species from Chandra Valley in Kangra district of Himachal Pradesh.

In having whiter thallus and K+ yellow reaction it is similar to *L. lactea* Flörke ex Schaer., which can be differentiated by the presence of norstictic acid and having more neatly arranged angular areoles.

Lecidea paratropoides Müll. Arg. Flora 57: 348 (1874). **Fig. 5**

Thallus indistinct (not developed); prothallus not seen; apothecia rounded to angular, crowded, 0.5–1.0 mm in diam.; margin thick, entire, black; disc plane to slightly convex, black, epruinose; epihymenium olive green to dark green 12–17 µm; hymenium colourless, 40–65 µm high; hypothecium brown to dark brown 40–50 µm high; excipulum colourless. Asci 26–30 x 10–15 µm; ascospores ellipsoid, 7–9 x 3.5–5 µm.

Thallus K–, KC–, C–, PD–: no lichen substances detected with TLC.

> SPECIMEN EXAMINED–UTTARANCHAL: Chamoli district, Kedarnath hill side, on east and north of the temple, 3600–3700 m on boulders, Dange 76-293 (LWU-LWG); Almora (now Bageshwar) district, Pindari Glacier, on ridge of moraine, 3940 m, Awasthi 7716 (LWG – AWAS).

The species is widely distributed in the alpine regions of southern Europe and Central Asia. In India it has a restricted distribution in the western Himalayas and first reported by Hertel (1977) from Pindari glacier in Uttaranchal.

L. promiscens Nyl., which is close to this species, can be differentiated by having larger (9–12 x 3–5 µm) ascospores and blackish-brown hypothecium.

Lecidea plana (J. Lahm) Nyl. Flora 55: 552 (1872). **Fig. 6**
– *Lecidella plana* J. Lahm in Körber, Parerga Lich. 211 (1861).

Thallus absent or thin, white, granular to cracked, areolate, areoles flat, white to grey; prothallus indistinct; apothecia adnate 0.4–1.2 mm diam., black; margin thin, entire or more or less sinuous; disc flat to somewhat convex; epihymenium deep green, intensifying in K; hymenium colourless, 40–50 µm high; hypothecium colourless, 50–80 µm high; exciple colourless. Asci clavate, 30–40 x 8–14 µm; ascospores 8–14 x 3–6 µm, ellipsoid.

Thallus and medulla K–, C–, KC–, PD–; planaic acid in TLC.

> SPECIMENS EXAMINED–HIMACHAL PRADESH: Lahul Spiti district, Sissu, 3100 m, on rocks, Upreti and Chatterjee 03-001706 (LWG). **JAMMU AND KASHMIR:** Leh district, Khardungla Pass, 4700 m, on rocks, Upreti and Chatterjee 03-001806/B (LWG). **UTTARANCHAL:** Chamoli district, Badrinath, East of Temple, way to Devdarshani, 3150 m, on boulders, Dange 76-814 (LWU-LWG).

The species is distributed in Europe, North America, Japan, Australia and is a new record for Indian lichen flora. It preferably grows on coarse to grained siliceous rocks in alpine habitats.

L. plana is closely related to *L. lithophila* (Ach.) Ach., in anatomy and morphology.

L. plana can be distinguished by having smaller ascospores, greenish epithecium, lower hymenium and adnate, purely black apothecia.

Lecidea tessellata Flörke Deutsche Lichenen, no. 64 (1819). **Fig. 7**

Thallus cracked areolate, chalky white to grey, areolate, plane, medulla I+ blue; apothecia subimmersed, appressed to adnate, black, 0.5–1.8 (–2.0) mm diam.; disc smooth, plane, rounded in young apothecia, convex, variously irregular in mature ones, with sometimes thin, white pruinose; epihymenium brownish-green to blackish green; hymenium colourless, 40–60 µm high; hypothecium pale brown, 30–40 µm high; excipulum blackish-green externally, colourless internally. Asci clavate, 30–50 x 8–14 µm, ascospores ellipsoid, 7–9 x 5–6 µm.

Thallus and medulla K–, C–, PD–; confluentic acid in TLC.

> **Specimens examined–UTTARANCHAL:** Chamoli district, Badrinath east of Temple, way to Devdarshani, 3150 m, on boulders, Dange 76-794 (LWU-LWG). **HIMACHAL PRADESH:** Lahul Spiti district, Keylong, 2040 m, on exposed rocks, Upreti (LWG).

This species is widely distributed in Afghanistan, Russian Asia, China, Nepal, Europe and North America. It is a new record for Indian lichen flora and known only from the alpine Western Himalayas at an altitude of 3450 m.

In the presence of white thallus, it is similar to *L. lapicida* and *L. confluescens* Nyl. *L. lapicida* can be distinguished by having K+ yellow reaction (stictic and constictic acid) while *L. confluescens* has much bigger (12–14 x 5–7 µm) ascospores and I– reaction in the medulla.

Acknowledgements

We are grateful to Dr. R. Tuli, Director, NBRI for providing necessary laboratory facilities, to Drs. Gerhard Rambold, Christian Printzen and Teuvo Ahti for their valuable comments on the manuscript.

Literature Cited

Awasthi DD. 1963. Some noteworthy additions to the lichen flora of India and Nepal. Univ. Colorado Studies. Ser. Biol. 10: 28-36.

Awasthi DD. 1991. A key to the microlichens of India, Nepal and Sri Lanka. Biblioth. Lichenol. 40: 1-337.

Awasthi DD. 2000. Lichenology in Indian Subcontinent. Bishen Singh Mahendra Pal Singh, Dehra Dun, India

Culberson CF. 1972. Improved conditions and new data for the identification of lichen products by a standard thin layer chromatographic method. J. Chromat. 97: 107-108.

Hertel H. 1977. Gesteinsbewohnende Arten der Sammelgattung *Lecidea* (Lichenes) aus Zentral, Ost-, und Südasien. Khumbu Himal 6(3): 145-378.

Magnusson AH. 1940. Lichens from Central Asia I. In Repts. Sci. Exped. NW Prov. China (the Sino-Swedish Expedition). (Ed. Hedin, S.). 13, XI. Botany, 1: 1-168. Aktiebolaget Thule\ Stockholm. Pp. 1-168.

Poelt J. 1961. Flechten aus dem N.W. Karakorum in Rahmen der Deutschen Karakorum-Expedition 1959, von F. Lobbichler und Dr. Schneider gesammelt. Mitteil. Bot. Staatssamml. München 4: 83-94

Schneider G. 1980 ('1979'). Die Flechtengattung *Psora* sensu Zahlbruckner. Biblioth. Lichenol. 13: 1-129.

Sinha GP, Singh KP. 1987. Foliicolous lichens from Nagaland, India. Geophytology 17(2): 174-185.

Timdal E. 1984. The delimitation of *Psora* (*Lecideaceae)* and related genera, with notes on some species. Nordic J. Bot. 4: 525-540.

Walker FJ, James PW. 1980. A revised guide to microchemical technique for the identification of lichen products. Bull. Brit. Lich. Soc. 46: 13-29.

MYCOTAXON

Volume 95, pp. 331–334 January–March 2006

Contributions to the macrofungi of Bolu and Düzce Provinces, Turkey

Dursun Yağiz[1], Ahmet Afyon[1],
Muhsin Konuk[2] & Stephan Helfer[3]

dyagiz@selcuk.edu.tr aafyon@selcuk.edu.tr
mkonuk@aku.edu.tr s.helfer@rbge.org.uk

[1]Selcuk University, Education Faculty
42099, Meram, Konya-TURKEY

[2]Afyon Kocatepe University, Faculty of Art and Science
Afyonkarahisar-TURKEY

[3]Royal Botanic Garden Edinburgh
20A Inverleith Row, Edinburgh EH3 5LR, U.K.

Abstract—This study was based on specimens of macrofungi collected from Bolu and Düzce provinces between 1998 and 2004. According to the field and laboratory results, 277 taxa belonging to 44 families (7 families of *Ascomycetes* and 37 of *Basidiomycetes*) have been identified.

The full checklist is available at http://rbg-web2.rbge.org.uk/mycotaxon/56.pdf

Key words—macromycota, flora, Western Black Sea Region

Introduction

This research was carried out in Bolu and Düzce provinces of Turkey which are situated in the Western Black Sea Region (Figure 1). These provinces are placed in squares A3-A4 according to the floristic square system (Davis 1965-1985). In the study area mainly the second type of oceanic rain fall regime occurs (Akman 1990). But in some parts (e.g., in the west and southwest), it also displays continental climatic features.

One checklist for macrofungi of Turkey was produced by Sesli & Denchev (2005). According to this, there was only one previous study in the area (Sümer 1982). This account of wood decaying macrofungi and and a few other reports on other macrofungi are included here. The study area is covered mainly by natural forest due to the prevailing climatic and edaphic conditions and limited timber extraction. The canopy vegetation of the study area is composed of mainly coniferous and broadleaved trees. The research area therefore is an ideal habitat for mycological studies. This paper presents the most up-to-date and extensive list of macrofungi of Bolu and Düzce provinces.

Materials and Methods

Specimens were collected from the research area (Fig. 1) during field trips between 1998 and 2004. The morphological and ecological characters of the specimens were recorded *in situ* and their microscopic features determined in the laboratory. The diagnoses of the taxa were carried out with the help of Marchand (1971-1986), Phillips (1981), Moser (1983), Michael et al. (1983-1988), Breitenbach & Kränzlin (1984-1991) and Dähncke (1993). Taxa and their authors were given according to an amended CABI Bioscience electronic version (http://www.indexfungorum.org/Names/Names.asp). The specimens are kept at Selcuk University, Education Faculty Herbarium (KNYA).

Results

In our study, 277 species were recovered belonging to 44 families. The distribution of the species and their families are: *Dermataceae* 1, *Discinaceae* 1, *Helvellaceae* 4, *Rhizinaceae* 1, *Morchellaceae* 7, *Pezizaceae* 2, *Pyronemataceae* 1, *Agaricaceae* 14, *Albatrellaceae* 1, *Auriscalpiaceae* 1, *Bankeraceae* 3, *Bolbitiaceae* 11, *Boletaceae* 9, *Cantharellaceae* 1, *Clavariaceae* 1, *Clavulinaceae* 1, *Coprinaceae* 5, *Cortinariaceae* 15, *Dacrymycetaceae* 2, *Entolomataceae* 5, *Fomitopsidaceae* 1, *Ganodermataceae* 2, *Geastraceae* 4, *Gomphaceae* 2, *Gomphidiaceae* 2, *Hydnaceae* 1, *Hydnangiaceae* 1, *Hygophoropsidaceae* 1, *Hymenochaetaceae* 3, *Lycoperdaceae* 8, *Marasmiaceae* 16, *Paxillaceae* 1, *Phallaceae* 2, *Pleurotaceae* 1, *Pluteaceae* 11, *Polyporaceae* 10, *Ramariaceae* 4, *Rhizopogonaceae* 2, *Russulaceae* 38, *Stereaceae* 1, *Strophariaceae* 10, *Suillaceae* 4, *Tremellaceae* 2, and *Tricholomataceae* 64. Their collection dates, localities, habitats, collectors' names and herbarium numbers are given in the web version of the paper: http://rbg-web2.rbge.org.uk/mycotaxon/56.pdf .

Discussion

277 species of macrofungi were collected from the study area (Bolu and Düzce provinces, located in the western Black Sea Region of Turkey). 17 of them belonged to Ascomycotina and 260 to Basidiomycotina. Sümer (1982) reported 102 wood decaying macrofungi from the study area. There are no other reports related to our study area known to the authors. Öder (1986) reported 17 poisonous macrofungi and 14 edible taxa from the East Black Sea Region (Öder 1988). Some reports from neighbouring regions of the study area were: Afyon et al. (2000) 62 taxa from Bartın; Afyon & Konuk (2002) 77 taxa from Zonguldak; Afyon et al. (2004) 170 taxa from Sinop; Yagız et al. (2005) 121 taxa from Karabük, and Afyon et al. (2005) 80 wood decaying macrofungi from Western Black sea region. When comparing taxa given from the neighbouring areas, the present study gives a very high number of recorded taxa. Bolu seems to be the richest area for its macrofungi species when compared to the other parts of Turkey. A diversity of habitats may be the cause for this abundance of taxa. The five most species-rich families and their species numbers are *Tricholomataceae* 64, *Russulaceae* 38, *Marasmiaceae* 16, *Cortinariaceae* 15 and *Agaricaceae* 14. These 5 families include 147 species and their ratio is 53% when compared to total numbers of the taxa diagnosed.

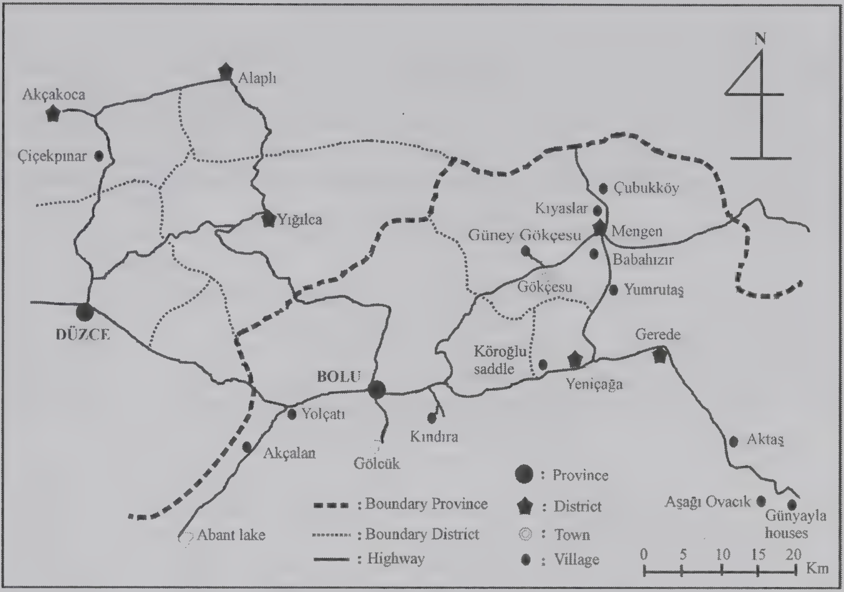

N
Alaplı
Akçakoca
Çiçekpınar
Yığılca
Çubukköy
Kıyaslar
Mengen
Güney Gökçesu
Babahızır
Gökçesu
Yumrutaş
DÜZCE
Gerede
Köroğlu saddle
Yeniçağa
BOLU
Yolçatı
Kındıra
Akçalan
Gölcük
Aktaş
: Province
: Boundary Province
: District
Aşağı Ovacık
Günyayla houses
Abant lake
: Boundary District
: Town
: Highway
: Village
0
5
10
15
20
Km

Figure 1: Map of the study area.

Acknowledgements

We would like to thank TÜBİTAK (The Scientific and Technical Research Council of Turkey) (TBAG-1659) and Selçuk University Scientific Research Project Commission (BAP: E.F/065) for supporting this study financially. Thanks are also due to Profs M. Isiloglu and R. Watling for reviewing the paper and for helpful comments.

Literature Cited

Afyon A, Konuk M, Yağız D. 2000. Bartın Yöresi Makrofungusları Üzerine Bir Araştırma. S. Ü. Eğitim Fak Fen Bilimleri Derg 8/2: 77-86.

Afyon A, Konuk M, Yağız D. 2004. Macrofungi of Sinop Province. Turk J Bot 28:351-360.

Afyon A, Konuk M, Yağız D, Helfer S, 2005. A study of wood decaying macrofungi of the western Black Sea Region, Turkey. Mycotaxon 93: 319-322.

Akman Y. 1990. İklim ve Biyoiklim. Ankara: Palme Yayın Dağıtım.

Breitenbach J, Kränzlin F. 1984-1991. Fungi of Switzerland.Vol.1-3. Lucerne: Verlag Mycologia.

Davis PH (ed.). 1965-1985. Flora of Turkey and East Aegean Islands. Vol. 1-9, Edinburgh Univ. Press.

Dähncke RM. 1993. 1200 Pilze. Aarau: AT Verlag.

Kirk PM, et al. 2004. Authors of fungal names. CABI Bioscience, Wallingford. Elecronic version: http://www.speciesfungorum.org/AuthorsOfFungalNames.htm

Marchand A. 1971-1986. Champignons du Nord et du Midi. Tome. 1-9. Perpignan: Soc. Mycol. Des Pyrénées Méditerr.

Michael E, Hennig B, Kreisel H. 1983-1988. Handbuch für Pilzfreunde. Bd. 1-5. Stuttgart: Gustav Fischer Verlag.

Moser M. 1983. In: Gams H (ed.). Kleine Kryptogamenflora. Stuttgart: Gustav Fischer Verlag.

Öder N. 1986. Karadeniz Bölgesinde (Sinop-Artvin İlleri Arası) Yetişen Önemli Bazı Zehirli Mantarlar Üzerinde Taksonomik Araştırmalar. S. Ü. Fen-Edebiyat Fak Fen Derg 5: 87-104.

Öder N. 1988. Karadeniz Bölgesinde (Sinop-Artvin İlleri Arası) Yetişen Halkın Tanıdığı Bazı Önemli Yenen Mantarlar Üzerinde Taksonomik Araştırmalar. S. Ü. Fen-Edebiyat Fak Fen Derg 8: 215-257.

Phillips R. 1981. Mushrooms and other Fungi of Great Britain and Europa. London: Pan Books Ltd.

Sesli E, Denchev CM. 2005. Checklists of the myxomycetes and macromycetes in Turkey. Mycologia Balcanica 2: 119-160.

Sümer S. (1982). Batı Karadeniz Bölgesi Özellikle Bolu Çevresinde Bulunan Odun Tahripçisi Mantarlar. İst. Üniv. Yayınları, No:2907/312.

Yağız D, Afyon A, Konuk M. 2005. The Macrofungi of Karabük Province. Turk J Bot., 29:345-353.

MYCOTAXON

Volume 95, pp. 335–340 January–March 2006

First isolation of *Aphanomyces frigidophilus* (*Saprolegniales*) in Europe

Isabel Ballesteros, María P. Martín
& Javier Diéguez-Uribeondo

dieguez@ma-rjb.csic.es
Departamento de Micología, Real Jardín Botánico C.S.I.C.
Plaza Murillo 2, Madrid 28014, Spain

Abstract—*Aphanomyces frigidophilus* was isolated and described for the first time in Europe. The isolate was characterized by studying its ability to undergo repeated zoospore emergence, to parasitize crayfish, and to produce chitinase constitutively, and by sequencing the internal transcribed spacer of nuclear ribosomal DNA (ITS1+5.8S+ITS2). The physiological properties studied differed from those of the "crayfish plague" parasite, *A. astaci*, but they were similar to those of saprobiotic *Aphanomyces* species. The ITS nrDNA sequence obtained from this isolate corresponded to *A. frigidophilus*.

Key words—*Aphanomyces astaci*, crayfish, conservation, rDNA, taxonomy

Introduction

The genus *Aphanomyces* de Bary belongs to the order *Saprolegniales* (Oomycetes) and comprises ca 30 species. Many of the species of this group have a saprobiotic mode of life, living on decayed animals and plant remains. A few species are detrimental parasites and responsible for economically important diseases affecting agriculture and aquaculture crops, as well as wildlife populations of freshwater animals (Papavizas & Ayers 1974, Söderhäll & Cerenius 1999). *Aphanomyces frigidophilus* Kitanch. & Hatai is a recently described species, which so far has only been found in salmonids eggs in Japan (Kitancharoen & Hatai 1997, 1998). Specimens resembling *A. frigidophilus* have been described in fish eggs in Poland (Czeczuga et al. 2004a, b, 2005). However, no isolations have been done to confirm the presence of this species in Europe or to study the molecular relatedness of European strains to the Japanese reference isolate of *A. frigidophilus*.

The taxonomy of *Aphanomyces* is largely based on the morphological characters of their sexual structures. However, the main taxonomic problems when describing species of this genus are: (i) that no reference isolates or cultures exist for several of the described species, and (ii) that many isolates, especially those of animal parasitic species are sterile;

consequently, species identification is largely based on their ability to parasitize their host, and a number of physiological properties.

The recent application of molecular tools to the genus *Aphanomyces* has helped identifying sterile isolates either in culture or clinical samples (Oidtmann et al. 2002, 2004; Phadee et al. 2004, Royo et al. 2004, Vandersea et al. 2006) and even defining new species (Royo et al. 2004). In this article, we have studied the internal transcribed spacer of nuclear ribosomal DNA sequences (ITS nrDNA) to identify a sterile isolate obtained from a mass mortality of indigenous crayfish, *Austropotamobius pallipes* (Lereboullet 1858) that occurred in the Central Iberian Peninsula region. Because the isolate exhibited different physiological properties from the crayfish plague fungus, *A. astaci* Schikora, it was decided to explore this isolate more carefully and obtain ITS sequence data, the results of which are described in this contribution.

Materials and Methods

Dead crayfish, *Austropotamobius pallipes*, were collected in the river Tajuña, Guadalajara (Spain). The isolation procedure was done from pieces of sub-abdominal cuticle taken from the crayfish as described by Cerenius et al. (1988). The isolate was maintained on PG1-agar (Unestam 1965) and stored under the strain name SAP233 in the culture collection of the Real Jardín Botanico de Madrid. Morphological characters of asexual structures and measurements were made microscopically on material mounted in water. Light micrographs were captured using a QImaging Micropublisher digital camera (QImaging, Burnaby, BC, Canada) mounted on an Olympus BX51 compound microscope as described in Diéguez-Uribeondo et al. (2003).

Physiological properties: repeated zoospore emergence characteristic of parasitic *Aphanomyces species*, and constitutive production of chitinase, characteristic of *A. astaci*, were studied according to methods described by Cerenius & Söderhäll (1985), and Andersson & Cerenius (2002), respectively. Production of sexual structures was studied by growing the isolate in corn meal agar, hemp seed or snake skin. In corn meal agar the isolate was paired with representative strains of the four genotypes of *A. astaci* (Huang et al. 1994, Diéguez-Uribeondo et al. 1995). The cultures were maintained and regularly checked for the production of sexual structures during a one month period.

The ability to infect crayfish was tested by following the method described in Cerenius et al. (1988). After finishing the experiment pieces of sub-abdominal cuticle were examined under the microscope for the presence of hyphae.

For DNA extraction, mycelium was grown as drop cultures (Cerenius & Söderhäll 1985), and from them, genomic DNA was extracted using an E.Z.N.A.-Fungi DNA miniprep kit (Omega Biotek, Doraville, USA). DNA fragments containing internal transcribed spacers ITS1 and ITS2 including 5.8S, were amplified with primer pair ITS5/ITS4 (White et al. 1990) primers as described in Martín et al. (2004). Nucleotide BLASTN searches with option Standard nucleotide BLAST of BLASTN 2.6 were used to compare the sequence obtained against the sequences in the National Center of Biotechnology Information (NCBI) nucleotide databases. The new consensus sequence has been deposited in the EMLB database with the accession Number 281399.

Results

The isolate obtained exhibited thin hyphae with rounded hyphal tips, and with a hyphal diameter that ranged from 5 to 7 μm (Fig. 1a). The isolate produced sporangia with a single row of primary spores. The primary spores were eventually released and encysted at the hyphal tip forming spore-balls characteristic for the genus *Aphanomyces* (Fig. 1b). No oogonia or antheridia were seen in either individual cultures or in co-culture with the other isolates or with representive strains of four genetic groups of *A. astaci*. Thus the strains appeared to be sterile and lack sexual reproduction.

The encysted zoospores did not undergo repeated zoospore emergence and instead germinated. No chitinase activity was detected in the culture filtrates of the new isolate, while reference *A. astaci* strains produced high level of extracellular chitinase (Fig. 1 c, d, e). Crayfish challenged with zoospores of the *Aphanomyce*s sp. isolate did not die under the experimental conditions. However, addition of zoospores of the reference isolates of *A. astaci* always caused 100% mortality of crayfish.

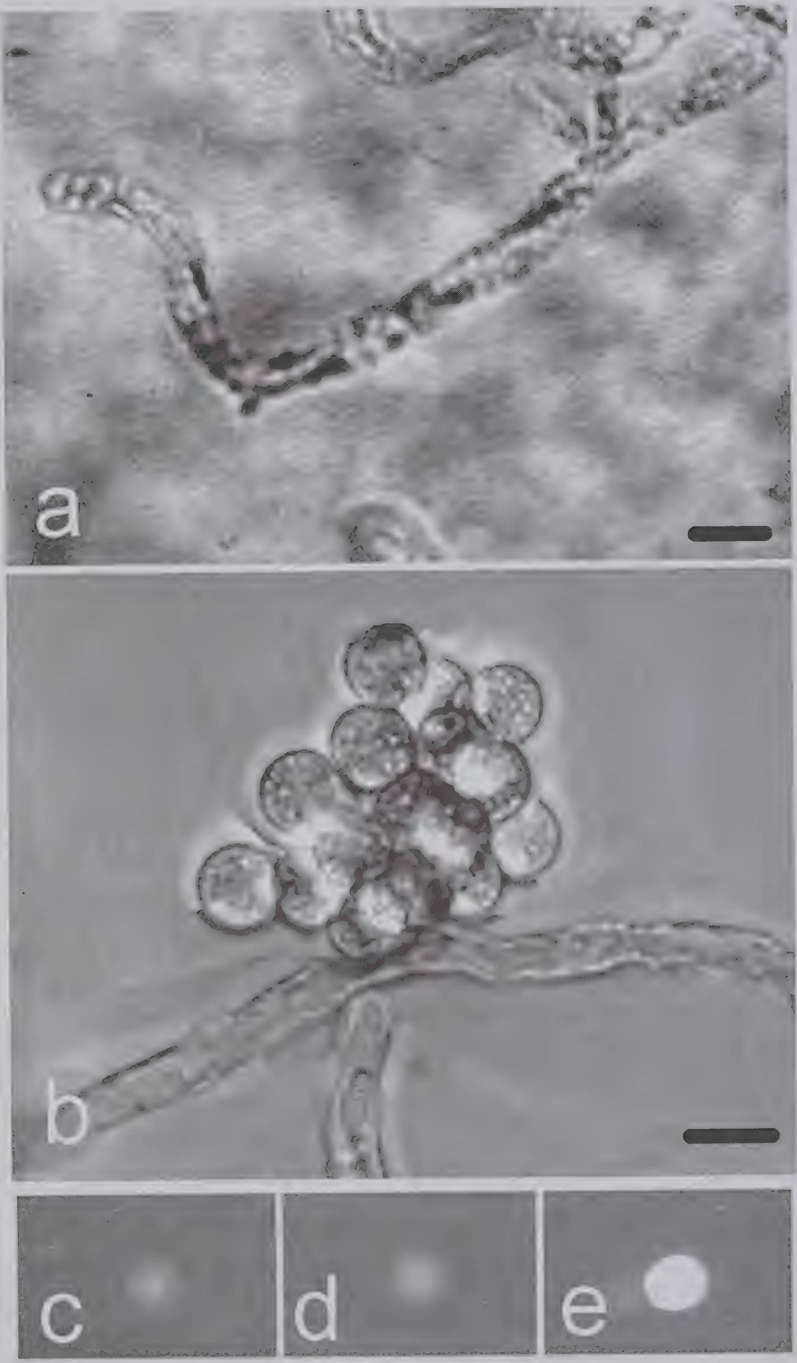
The blast search of the sequence of the isolate showed 99% similarity (the sequence differed in one base for ITS1 and four bases in ITS2 out of the 673 bases compared) to Genbank sequence AY647192 corresponding to strain NJM9500 of *A. frigidophilus* directly submitted by Phadee et al. (2004), and 95% (the sequence differed in 22 bases for ITS1 and 10 bases in ITS2 out of the 614 bases compared to Genbank sequences of *A. astaci.*

Discussion

Previous studies have shown that analyses of the ITS nrDNA represents a useful tool for differentiating individual saprolegniaceous species (Molina et al. 1995, Leclerc 2000, Oidtmann et al. 2004, Phadee et al. 2004). Due to the lack of sexual structures of the studied isolate, we carried out Genbank sequence comparisons of the two ITS nrDNA for species identification. The results indicated the sequence of our isolate corresponded to the species, *Aphanomyces frigidophilus*. Thus, this study represents the first isolation of *A. frigidophilus* in Europe and the first description of this species growing in a different substrate from salmonid eggs.

Interestingly, this isolate was growing in crayfish cuticle and associated to a mass mortality of indigenous European species of freshwater crayfish. These episodes are generally caused by the "crayfish plague" parasite *Aphanomyces astaci*. This parasite is considered among the 100 worst invasive species (Global Invasive Species Database 2005) and is responsible for the dramatic decline of the indigenous European freshwater crayfish species, which are currently endangered in Europe and at risk of extinction in the Iberian Peninsula (Diéguez-Uribeondo et al. 1997a, b, Söderhäll & Cerenius 1999). Current attempts to develop and improve techniques for rapid and accurate identification of this economically important parasitic species need to take into account the existence of closely related species, such as *A. frigidophilus*, in Europe.

Regarding the possible parasitic abilities of *A. frigidophilus*, our results indicate that its physiological properties are characteristic of a saprobiotic and/or opportunistic pathogen. Thus, the isolate exhibited a low percentage of secondary cysts undergoing repeated zoospore emergence, and, therefore, it lacks a character that appears to be


a
b
c
d
e

related to parasitism in *Aphanomyces* species (Cerenius & Söderhäll, 1985). The failure to kill crayfish challenged with zoospores and to produce chitinase constitutively, are also characters of the specialized crayfish parasite, *A. astaci* (Cerenius et al. 1988; Söderhäll & Cerenius 1999, Andersson & Cerenius 2004).

Aphanomyces frigidophilus and *A. astaci* seem to be closely related species which may occur in the same host with different abilities to colonize it. Further studies on phylogenetic relationships among *Aphanomyces* species need to be carried out in order to more accurately establish species limits. Finally, the results of this work emphasized the need to carry out isolations for a correct identification and characterization of saprolegniaceous species.

Acknowledgements

We thank Dr. Fernando Alonso (Centro de Investigaciones Agrarias de Albadalejito, Cuenca, Junta Castilla-La Mancha, Spain) for providing us with crayfish, and Dr. Stuart Gelder (Department of Science, University of Maine at Presque Isle, Maine, USA) for revising the English text. This study was supported by the Project Flora Micológica Ibérica (REN2002-04068-C02-01GLO), which also supported Dr. Isabel Ballesteros with a postdoctoral fellowship. We also acknowledge referees Dr. Lage Cerenius (Department of Comparative Physiology, University of Uppsala, Sweden) and Dr. Gordon Beakes (Department of University of Newcastle) for revising this article.

Literature Cited

Andersson MG, Cerenius L. 2002. Analysis of chitinase expression in the crayfish plague fungus *Aphanomyces astaci.* Diseases of Aquatic Organisms **51**: 139-147.

Cerenius L, Söderhäll K, 1985. Repeated zoospore emergence as a possible adaptation to parasitism in *Aphanomyces*. Experimental Mycology **9**: 259-263.

Cerenius L, Söderhäll K, Persson M, Ajaxon R. 1988. The crayfish plague fungus, *Aphanomyces astaci* – diagnosis, isolation, and pathobiology. Freshwater Crayfish **7**: 131-144.

Czeczuga B, Kiziewicz B, Muszynska E. 2004a. Presence of zoospore fungus species on the eggs of whitefish from Lake Goldopiwo, Mazury Region. Medycyna Weterynaryjna **60**: 379-383.

Czeczuga B, Kiziewicz B, Godlewska A. 2004b. Zoosporic fungi growing on eggs of *Coregonus lavaretus holsatus* Thienemann, 1916 from Lake Wdzydze in Kaszuby. Polish Journal of Environmental Studies **13**: 355-359 2.

Czeczuga B, Bartel R, Kiziewicz B, Godlewska A, Muszynska E. 2005. Zoosporic fungi growing on the eggs of sea trout (*Salmo trutta* m. *trutta* L.) in river water of varied trophicity. Polish Journal of Environmental Studies **14**: 295-303.

Diéguez-Uribeondo J, Huang TS, Cerenius L, Söderhäll K. 1995. Physiological adaptation of an *Aphanomyces astaci* strain isolated from the freshwater crayfish *Procambarus clarkii.* Mycological Research **99**: 574-578.

Diéguez-Uribeondo J, Rueda-Díez A, Castién E, Bascones JC. 1997a. A plan of restoration for the native freshwater crayfish species, *Austropotamobius pallipes*, in Navarra. Bulletin Francais de la Peche et de la Pisciculture **347**: 625-637.

Fig. 1 *Aphanomyces frigidophilus.* **a)** Hypha with a rounded tip growing within the cuticle of the freshwater crayfish *Austropotamobius pallipes.* **b)** "Spore balls" characteristic of the genus *Aphanomyces* (Bars 10 μm). **c–e)** Chitinase assay for production of chitinase constitutively during growth: **c)** negative control, without fungus; **d)** *A. frigidophilus* (negative); **e)** *A. astaci* (positive).

Diéguez-Uribeondo J, Temiño C, Múzquiz JL. 1997b. The crayfish plague fungus, *Aphanomyces astaci* in Spain. Bulletin Francais de la Peche et de la Pisciculture **347**: 753-763.

Diéguez-Uribeondo J, Förster H, Adaskaveg JE. 2003. Digital image analysis of internal light spots of appressoria of *Colletotrichum acutatum*. Phytopathology **93**: 923-930.

Global Invasive Species Database. 2005. Information Infrastructure (NBII) and Invasive Species Specialist Group (ISSG). *Aphanomyces astaci*. Available at: <http://www.issg.org/database/species/ecology.asp?si=107&fr=1&sts=sss>

Huang TS, Cerenius L, Söderhäll K. 1994. Analysis of the genetic diversity in crayfish plague fungus, *Aphanomyces astaci*, by random amplification of polymorphic DNA assay. Aquaculture **26**:1-10.

Kitancharoen N, Hatai K. 1997. *Aphanomyces frigidophilus* sp. nov. from eggs of Japanese char, *Salvelinus leucomaenis*. Mycoscience 38: 135-140.

Kitancharoen N, Hatai K. 1998. Some biochemical characteristics of fungi isolated from salmonid eggs. Mycoscience **39**: 249-255.

Leclerc MC., Guillot J, Deville M. 2000. Taxonomic and phylogenetic analysis of Saprolegniaceae (Oomycetes) inferred from LSU rDNA and ITS sequence comparisons. Antonie van Leeuwenhoek 77: 369-377.

Martín MP, Raidl S, Tellería MT. 2004. Molecular analysis confirm the relationship between *Stephanospora caroticolor* and *Lidtneria trachyspora*. Mycotaxon **90**: 133-140.

Molina FI., Jong SC., MA, G. 1995. Molecular characterization and identification of *Saprolegnia* by restriction analysis of genes coding for ribosomal RNA. Antonie van Leeuwenhoek **68**: 65-74.

Oidtmann B, Bausewein S, Hölzle L, Hoffmann R, Wittenbrink M. 2002. Identification of the crayfish plague fungus *Aphanomyces astaci* by polymerase chain reaction and restriction enzyme analysis. Veterinary Microbiology **85**: 183–194.

Oidtmann B, Schaefer N, Cerenius L, Söderhäll K, Hoffmann RW. 2004. Detection of genomic DNA of the crayfish plague fungus *Aphanomyces astaci* (Oomycete) in clinical samples by PCR. Veterinary Microbiology **100**: 269-282.

Papavizas GC, Ayers A. 1974. *Aphanomyces* species and their root diseases in pea and sugar beet. U.S. Department of Agriculture Technical Bulletin no **1485**.

Phadee P, Kurata O, Hatai K, Irono I, Aoki T. 2004. Detection and identification of the fish-pathogenic *Aphanomyces piscicida* using polymerase chain reaction (PCR) with species-specific primers. Journal of Aquatic Animal Helath **16**: 220-230.

Royo F, Andersson MG, Bangyeekhun E, Muzquiz JL, Söderhäll K, Cerenius L. 2004. Physiological and genetic characterisation of some new *Aphanomyces* strains isolated from freshwater crayfish. Veterinary Microbiology **104**: 103-112.

Söderhäll K, Cerenius L. 1999. The crayfish plague fungus: history and recent advances. Freshwater Crayfish **12**: 11-35.

Vandersea MW, Litaker RW, Yonnish B, Sosa E, Landsberg JH, Pullinger C, Moon-Butzin P., Green J., Morris JA, Kator H, Noga EJ, Tester PA. 2006. Molecular assays for detecting *Aphanomyces invadans* in ulcerative mycotic fish lesions Applied and Environmental Microbiology 72: 1551-1557.

Unestam T. 1965. Studies on the crayfish plague fungus, *Aphanomyces astaci* I. Some factors affecting growth in vitro. Physiologia Plantarum **18**: 483-505.

White TJ, Bruns T, Lee S, Taylor JW. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In PCR Protocols. A guide to methods and applications (eds. Innis MA, Gelfand DH, Sninsky JJ, White TJ.), pp. 315-322. Academic Press Inc., San Diego, CA.

Nomenclatural novelties proposed in Mycotaxon 95

Arthrobotrys multisecundaria W.F. Hu & K.Q. Zhang, p. 182
Brachydesmiella brasiliensis R.F. Castañeda, Gusmão & Heredia, p. 262
Brachydesmiella obclavata R.F. Castañeda, Gusmão & Saikawa, p. 265
Calostoma zanchianum Baseia & Calonge, p. 114
Coccomyces taiwanensis C. L. Hou, R. Kirschner & Chee J. Chen, p. 73
Cookeina colensoiopsis Iturriaga & Pfister, p. 151
Doassansiopsis tomasii Vánky, p. 46
Embellisia oxytropis Q. Wang, Nagao & Kakish., p. 25
Geastrum hirsutum Baseia & Calonge, p. 302
Heterodoassansia hygrophilae (Thirum.) Vánky, p. 56
Hyphodontia tubuliformis Sheng H. Wu, p. 185
Kalmusia amphiloga O.E. Erikss. p. 67
Lycoperdon ovalicaudatum D. Bisht, J.R. Sharma & Kreisel, p. 92
Marasmius dimorphus C. Puccin. & Capelari, p. 298
Marasmius pseudosetosus C. Puccin. & Capelari, p. 295
Meliola thalliformis var. *major* D.J. Soares & R.W. Barreto, p. 202
Microbotryum afromontanum Vánky, p. 50
Morchella deqinensis Shu H. Li, Y.C. Zhao, H.M. Chai & M.H. Zhong, p. 319
Morchella meiliensis Y.C. Zhao, Shu H. Li, H.M. Chai & M.H. Zhong, p. 321
Paecilomyces verticillatus Z.Q. Liang. Z. Li & Y.F. Han et al, p. 134
Parmotrema sorediosulphuratum Eliasaro & Donha, p. 242
Parmotrema superaguiense Donha & Elisaro, p. 243
Pilocintractia adrianae Vánky, p. 54
Repetophragma fasciata (R.F. Castañeda) Castañeda, Gusmão & Saikawa, p. 269
Repetophragma filifera (Piroz.) R.F. Castañeda, Gusmão & Heredia, p. 269
Russula appendiculata K. Das, S.L. Mill. & J.R. Sharma, p. 210
Russula dhakuriana K. Das, J.R. Sharma & S.L. Mill., p. 208
Russula mayawatiana K. Das, S.L. Mill. & J.R. Sharma, p. 206
Russula natarajanii K. Das, J.R. Sharma & Atri, p. 272
Russula puellaris var. *atrii* K. Das, S.L. Mill. & J.R. Sharma, p. 211
Russula siamensis Yomyart, Piapukiew, Watling, Whalley & Sihan., p. 249
Sporisorium andropogonis-chinensis Vánky, p. 5
Sporisorium andropogonis-eucomi Vánky, p. 5
Sporisorium andropogonis-gabonensis Vánky, p. 7
Sporisorium andropogonis-pumili Vánky, p. 7
Sporisorium andropteri (Zambett.) Vánky, p. 59
Sporisorium clintonianum Vánky, p. 30
Sporisorium distachyum Vánky, p. 12
Sporisorium ingoldii Vánky, p. 50

Sporisorium livingstoneanum Vánky, p. 17
Sporisorium sanctae-catharinae (Zundel) Vánky, p. 21
Sporisorium scholzii Vánky, p. 22
Sporisorium semisagittatum (Thirum. & Pavgi) Vánky, p. 60
Sporisorium sorghastri (Zundel) Vánky, p. 30
Sporisorium zilligii (Zundel) Vánky, p. 25
Tricholoma borgsjoeënse Jacobsson & Muskos, p. 197
Tricholoma lavendulophyllum F.Q. Yu, p. 306
Tuber quercicola J.L. Frank, Southworth & Trappe, p. 232
Tuber whetstonense J.L. Frank, Southworth & Trappe, p. 235
Ustanciosporium virginianum Vánky, p. 48
Ustilago gabonensis Vánky, p. 39
Ustilago jardineae (Zambett.) Vánky, p. 39
Ustilago penniseti-purpurei Vánky, p. 44
Ustilago pentaschistidis Vánky, p. 42
Ustilago trichogena Vánky, p. 54

Author Index—Volume 95

Abarca, Gabriela Heredia, see **Castañeda-Ruiz & al.**

Adler, Mónica T., Susana Calvelo & John A. Elix. The distinction between *Menegazzia cincinnata* and *M. valdiviensis (Parmeliaceae)*. 95: 217–228. 2006.

Afyon, Ahmet, see **Yağız & al.**

Andreev, M.P., see **Upreti & al.**

Aslan, Alı, see **Yazıcı & Aslan**

Atri, N.S., see **Das & al.**

Ballesteros, Isabel, María P. Martín & Javier Diéguez-Uribeondo. First isolation of *Aphanomyces frigidophilus* (*Saprolegniales*) in Europe. 95: 335-340. 2006.

Barreto, Robert Weingart, see **Soares & al.**

Baseia, Iuri G., Vagner G. Cortez & Francisco D. Calonge. Rick´s species revision: *Mitremyces zanchianus* versus *Calostoma zanchianum*. 95: 113–116. 2006.

Bisht, Dipika, J.R. Sharma, Hanns Kreisel & Kanad Das. A new species and a new record of *Lycoperdaceae* from India. 95: 91-96. 2006.

Calonge, F.D., see **Baseia & Calonge**

Calonge, Francisco D., see **Baseia & al.**

Calvelo, Susana, see **Adler & al.**

Capelari, see **Puccinelli & Capelari**

Caretta, Giuseppe, Solveig Tosi, Eduardo Piontelli & G.S. de Hoog. *Phialophora sessilis,* a lithobiont fungus. 95: 281-284. 2006.

Castañeda-Ruiz, Rafael F., Luís Fernando Pascholati Gusmão, Gabriela Heredia Abarca & Masatoshi Saikawa. Some hyphomycetes from Brazil. Two new species of *Brachydesmiella,* two new combinations for *Repetophragma,* and new records. 95: 261–270. 2006.

Chai, Hong-Mei, see **Li & al.**

Chen, Chee-Jen, see **Hou & al.**

Coronado, Martha, see **Esqueda & al.**

Cortez, Vagner G., see **Baseia & al.**

Cunnington, James H., see **Hamilton & Cunnington**

Das, Kanad, J.R. Sharma & N.S. Atri. *Russula* in Himalaya 3: A new species of subgenus *Ingratula*. 95: 271–275. 2006.

Das, Kanad, S.L. Miller & J.R. Sharma. *Russula* in Himalaya 2: Four new taxa. 95: 205–215. 2006.

Das, Kanad, see **Bisht & al.**

de Hoog, G.S., see **Caretta & al.**

Diéguez-Uribeondo, Javier, see **Ballesteros & al.**

Donha, Cristine G. & Sionara Eliasaro. Two new species of *Parmotrema* (*Parmeliaceae,* Lichenized Ascomycota) from Brazil. 95: 241–245. 2006.

Eliasaro, Sionara, see **Donha & Eliasaro**

Elix, John A., see **Adler & al.**

Eriksson, Ove E. *Kalmusia amphiloga* comb. nov. on *Bambusa.* 95: 67–69. 2006.

Frank, Jonathan L., Darlene Southworth & James M. Trappe. NATS truffle and truffle-like fungi 13: *Tuber quercicola* and *T. whetstonense*, new species from Oregon, and *T. candidum* redescribed. 95: 229–240. 2006.

Gleason, Mark L., see **Sun & al.**

Góes-Neto, Aristóteles, see **Lamrood & Góes-Neto**

Gusmão, Luís Fernando Pascholati, see **Castañeda-Ruiz & al.**

Hamilton, Andrew J. & James H. Cunnington. Calculating minimum sample sizes for taxonomic measurements: examples using Gäumann's *Peronospora* spore data. 95: 189–194. 2006.

Han, Yanfeng, see **Li & al.**

Helfer, Stephan, see **Yağız & al.**

Herrera, Teófilo, see **Esqueda & al.**

Hou, Cheng-Lin, Roland Kirschner & Chee-Jen Chen. A new species and new records of *Rhytismatales* from Taiwan. 95: 71–79. 2006.

Hu, Weifeng, Yan Li, Minghe Mo & Keqin Zhang. A new nematode-trapping hyphomycete of *Arthrobotrys.* 95: 181-184. 2006.

I.G. Baseia & F.D. Calonge. *Geastrum hirsutum:* a new earthstar fungus with a hairy exoperidium. 95: 301–304. 2006.

Iturriaga, Teresa & Donald H. Pfister. A monograph of the genus *Cookeina* (Ascomycota, *Pezizales, Sarcoscyphaceae*). 95: 137–180. 2006.

Jacobsson, S., S. Muskos & E. Larsson. *Tricholoma borgsjoeënse,* a new species from boreal coniferous forest in Fennoscandia. 95: 195–200. 2006.

Kakishima, Makoto, see **Wang & al.**

Kirschner, Roland, see **Hou & al.**

Knudsen, Kerry & James C. Lendemer. Changes and additions to the North American lichen mycota – V. Mycotaxon 95: 309–313. 2006.

Konuk, Muhsın, see **Yağız & al.**

Kreisel, Hanns, see **Bisht & al.**

Lamrood, Prasad & Aristóteles Góes-Neto. Taxonomic studies on Indian *Phellinus* s.l. species: parsimony analysis using morphological characters. 95: 117–131. 2006.

Larsson, E., see **Jacobsson & al.**

Lendemer, James C., see **Knudsen & Lendemer**

Li, Shu-Hong, Yong-Chang Zhao, Hong-Mei Chai & Ming-Hui Zhong. Two new species in the genus *Morchella* (*Pezizales, Morchellaceae*) from China. 95: 319–322. 2006.

Li, Yan, see **Hu & al.**

Li, Yu-ling, see **Wang & al.**

Li, Zhu, Yanfeng Han & Zongqi Liang. *Paecilomyces verticillatus,* a new species isolated from soil in China. 95: 133–136. 2006.

Liang, Zongqi, see **Li & al.**

Liu, Pei-Gui, see **Yu & al.**

Ma, Huining, see **Sun & al.**

Martín, María P., see **Ballesteros & al.**

Miller, S.L., see **Das & al.**

Mo, Minghe, see **Hu & al.**

Muskos, S., see **Jacobsson & al.**

Nagao, Hideyuki, see **Wang & al.**

Nayaka, S., see **Upreti & al.**

Parreira, Douglas Ferreira, see **Soares & al.**

Pérez-Silva, Evangelina, see **Esqueda & al.**

Pfister, Donald H., see **Iturriaga & Pfister**

Piapukiew, J., see **Yomyart & al.**

Piontelli, Eduardo, see **Caretta & al.**

Puccinelli, Carla & Marina Capelari. Two new species of *Marasmius* (Basidiomycota, *Marasmiaceae*) from Brazil. 95: 295–300. 2006.

Ryley, Malcolm J. Notes on the type, synonyms, and other specimens of the balansioid fungus, *Nigrocornus scleroticus*. 95: 97-112. 2006.

Saikawa, Masatoshi, see **Castañeda-Ruiz & al.**

Sánchez, Alfonso, see **Esqueda & al.**

Sharma, J.R., see **Bisht & al.**

Sharma, J.R., see **Das & al.**

Sharma, J.R., see **Das & al.**

Sihanonth, P., see **Yomyart & al.**

Soares, Dartanhã J., Douglas Ferreira Parreira & Robert Weingart Barreto. A new variety of *Meliola thalliformis* from Brazil. 95: 201–204. 2006.

Southworth, Darlene, see **Frank & al.**

Sun, Guangyu, Miao Zhang, Rong Zhang, Huining Ma & Mark L. Gleason. *Wallemia*, a genus newly recorded from China. 95: 277–280. 2006.

Tang, B.-H., T.-Z. Wei & Y.-J. Yao. Revision of *Termitomyces* species originally described from China. 95: 285–293. 2006.

Teófilo Herrera. Macromycetes of Pinacate and Great Altar Desert biosphere reserve, Sonora, Mexico. 95: 81–90. 2006.

Tosi, Solveig, see **Caretta & al.**

Trappe, James M., see **Frank & al.**

Upreti, D.K., S. Nayaka & M.P. Andreev. Notes on some species of the lichen genus *Lecidea* from India. 95: 323-330. 2006.

Vánky, Kálmán. Taxonomic studies on Ustilaginomycetes—26. 95: 1–65. 2006.

Wang, Hong-sheng, see **Wang & al.**

Wang, Qi, Hideyuki Nagao, Yu-ling Li, Hong-sheng Wang & Makoto Kakishima. *Embellisia oxytropis*, a new species isolated from *Oxytropis kansuensis* in China. 95: 255–260. 2006.

Wang, Yun, see **Yu & al.**

Watling, R., see **Yomyart & al.**

Wei, T.-Z., see **Tang & al.**

Whalley, A.J.S., see **Yomyart & al.**

Wu, Sheng-Hua. *Hyphodontia tubuliformis*, a new species from Taiwan. 95: 185-188. 2006.

Yağız, Dursun, Ahmet Afyon, Muhsın Konuk, Stephan Helfer. Contributions to the macrofungi of Bolu and Düzce Provinces, Turkey. 95: 331-334 2006.

Yao, Y.-J., see **Tang & al.**

Yazıcı, Kenan & Alı Aslan. Four new lichens from Turkey. 95: 315–318. 2006.

Yomyart, S., J. Piapukiew, R. Watling, A.J.S. Whalley & P. Sihanonth. *Russula siamensis:* a new species of annulate *Russula* from Thailand. 95: 247–254. 2006.

Yu, Fu-Qiang, Yun Wang & Pei-Gui Liu. *Tricholoma lavendulophyllum,* a new species from Yunnan, China. 95: 305–308. 2006.

Zhang, Keqin, see **Hu & al.**

Zhang, Miao, see **Sun & al.**

Zhang, Rong, see **Sun & al.**

Zhao, Yong-Chang, see **Li & al.**

Zhong, Ming-Hui, see **Li & al.**

Reviewers, Volume Ninety-four

The Editors express their appreciation to the following individuals who have, prior to acceptance for publication, reviewed one or more of the papers appearing in this volume.

Teuvo Ahti
Vladimír Antonín
André Aptroot
Narinder S. Atri
Gordon W. Beakes
Reinhard Berndt
Gregory Bonita
Irwin Brodo
Francisco D. Calonge
Susana Calvelo
Sharon A. Cantrell
Lori Carris
Lage Cerenius
Pedro Crous
Dennis E. Desjardin
Sionara Eliasaro
John A. Elix
Javier Etayo
Harry C. Evans
Alan M. Fryday
James H. Ginns
Gro Gulden
Ying-Lan Guo
Nils Hallenberg
Karen Hansen
Lennart Holm
V.G. Hosagoudar
Mustafa Isıloğlu
Peter Johnston
Jichuan Kang
Ken Katumoto
Henning Knudsen
Hanns Kreisel
Teresa Lebel
ShiDong Li
XingZhong Liu
Zuoyi Liu
Nils Lundqvist
Xin-Chang Luo
E.H.C. McKenzie
Steven Miller
David W. Minter
Gabriel Moreno
Eiji Nagasawa
Lorelei L. Norvell
Silvano Onofri
Breuss Othmar
Clark Ovrebo
Ian Pasco
David Pegler
Shaun Pennycook
Michael Priest
Christian Printzen
Gerhard Rambold
Peter Roberts
Wayne Robinson
Jack D. Rogers
Roger Shivas
Bin Song
Turner Sutton
Chris Triggs
Clara Urzi
Jukka Vauras
Roy Watling
Zhu L. Yang
Wen-ying Zhuang

ERRATA

Volume 92

p. 181, line 2	for: *abnormis*	read: *abnorme*
p. 214, line 15	for: *M.*	read: *Metarhizzium*
p. 243, Intro.line 10	for: *macrosporus*	read: *macrospora*
p. 244, line 9	for: *yungiensis*	read: *yungensis*
p. 264, line 5	for: [illegible overprint]	read: var. *robustum* MUCL
p. 302, 6th line from bot	for: *brogniartii*	read: *brongniartii*
p. 303, line 17	for: *A. ferrugineus*	read: *Ascobolus ferrugineus*
p. 304 & 305, legends	for: *gamundii*	read: *gamundiae*
p. 359, line 11	for: *chlamyfosporum*	read: *chlamydospora*
p. 384, 7th linn from bot	for: *Acroditys*	read: *Acrodictys*
p. 410, line 8	for: *Psecocercospora*	read: *Pseudocercospora*
p. 443, Abstr. line 8	for: *hydrocotyles, P.*	read: *hydrocotyles, P. spinulosa, P.*
—, *ibid.*	for: *achyrantis*	read: *achyranthis*
p. 448, line 8	for: *achyrantis*	read: *achyranthis*
p. 452, 5th line of tree	for: *spinulosus*	read: *spinulosa*
p. 456, line 19	for: *achyrantis*	read: *achyranthis*
p. 497, line 8	*for:* p. 385	*read:* p. 384
—, lines 9 & 10	*for:* p. 251	*read:* p. 252
—, lline 19	*for:* p, 224	*read:* p. 225
—, lline 33	*for:* p. 93	*read:* p. 92
p. 498, line 4 from bot	*for:* p. 410	*read:* p. 401

Volume 93

p. 379, line 4,	for: *aurelio@castillo@uah.es*	read: *aurelio.castillo@uah.es*

Volume 94

Front cover, last entry	*for:* Vamsaprija indica	read: *Vamsaprija indica*
p. ii, in Editorial Board	*for:* Sean Ross Pennycook	*read:* Shaun Ross Pennycook
p. iii, 7th line from bottom	*for:* Vamsaprija indica	read: *Vamsaprija indica*
p. 15, abstract, line 5	for: *C luteolus*	read: *C. luteolus*
p. 393, 5th from bottom	for: *Thyrostoma*	read: *Thyrostroma*
p. 400, Volume 93,	for: *aurelio.castillo.uah.es*	read: *aurelio.castillo@uah.es*

FROM THE *EDITOR-IN-CHIEF*

Color returns to MYCOTAXON

Volume 95 brings with it a welcome surprise: color. Over two decades have passed since color has graced the pages of two previous MYCOTAXON volumes, primarily due to the high printing cost. Fortunately, Iturriaga & Pfister and Frank, Southworth & Trappe were able to find sufficient funds to print their plates in full color in the current volume.

The results are stunning. You will find the photographs of *Cookeina* on page 141 and of *Tuber* on pages 233 & 237. MYCOTAXON thanks the authors for allowing us to demonstrate mycology's more beautiful nature.

Publication schedule for 2006

Due to a combination of unanticipated problems, MYCOTAXON is running behind schedule. Volume 95 was delayed by a series of computer and health set-backs, and Volume 96 is thinner than planned, primarily due to the number of manuscripts still in revision. We fully intend to bring you four volumes in 2006 and hope that we receive enough manuscripts to bring you the full number of pages promised in Volume 94. The good news is that the Editors are now completely caught up with their backlogs so that manuscript turn-around time is much quicker than usual. Volumes 96–98 will be sent as they fill, and the journal should be back on its normal quarterly schedule by year's end.

To our Authors

Authors are asked to download instructions and forms from our INSTRUCTIONS TO AUTHORS webpage on <www.mycotaxon.com> before preparing a paper for submission to our journal. An abbreviated set of instructions was also published in MYCOTAXON 94: 401–411 (2005/2006). All files are also available as Email attachments from the *Editor-in-Chief* <editor@mycotaxon.com> on request.

MYCOTAXON submission and publication preparation involves four steps:

Step 1—peer review: Authors send their formatted text document, illustration files, and Reviewer Guidelines + checklist to two experts for peer review. Peer reviewers must (a) return edited manuscripts and detailed comments to the authors and (b) Email completed checklist forms and brief comments to the *Editor-in-Chief* before authors submit their manuscripts for nomenclatural review.

Step 2—nomenclatural & French language review: After revising their manuscript following peer review recommendations, authors Email a revised master clone (containing footnotes, tables and captions but ***no*** illustrations) to the *Nomenclature Editor* at <PennycookS@LandcareResearch.co.nz>. The Email message must include the word 'Mycotaxon' on the subject line and list names **and** Email addresses of all peer reviewers. Manuscripts written in French should also be submitted to the *French Language Editor* <hennebertg@mbla.ucl.ac.be> at this time. Each Editor will return annotated files with a list of needed corrections to the authors and *Editor-in-Chief.*

Step 3—final submission: Authors Email/post the following to the *Editor-in-Chief*: a completed MYCOTAXON submission form; one manuscript PDF/MSWord file **or** printed copy showing all text, legends, tables, footnotes & graphics in place; a **body** text clone (and table/legend text clones when tables, footnotes, and legends are present), and artwork files. All materials ***must*** be correct so that the manuscript can be prepared for immediate publication if necessary. The *Editor-in-Chief* usually acknowledges receipt within two weeks, but acknowledgments of new submissions may slow near press deadlines or when the editorial office is closed for research.

Step 4—press preparation: The *Editor-in-Chief* combines all files together to produce a press-quality PDF. Entries for the Nomenclatural novelties and Author index pages are sent with the PDF to all coauthors for approval after conversion. Editorial errors are always corrected free of charge. Authors who request correction of errors present in original author-prepared files, however, must pay at least $10 per correction (minimum charge of $40) to cover editorial time and the production/delivery of an invoice. Payment arrangements of all fees should be made by writing the *Business Manager* <info@mycotaxon.com>

To our Readers

MYCOTAXON frequently updates its webpages and regularly posts abstracts, table of contents, indices, distributional checklists, and revised instructions and forms on its website. We invite all of you to browse our website frequently for updated journal and other interesting mycological news.

www.mycotaxon.com

MYCOTAXON is published quarterly during the periods of January–March, April–June, July–September, and October-December by **MYCOTAXON, LTD.**, 316 Richard Pl., Ithaca, NY 14850–0264. USPS Publication # 16-121, ISSN # 0093-4666. Periodical postage paid at Ithaca, NY, and at additional mailing offices. Subscription rates for 2006: In U.S. and possessions, one year, $330; reduced rate for personal subscribers, one year, $150. Foreign subscriptions, add $40 for IMEX air mail.

POSTMASTER!
Send address changes to **MYCOTAXON, LTD.**
P.O. Box 264, Ithaca, NY 14851-0264 USA

RECENT BOOKS FROM MYCOTAXON

Prices include surface mail shipment (*for more books see* **www.mycotaxon.com/books.html**)
Mycotaxon, Ltd., P. O. Box 264, Ithaca, NY 14851-0264, U.S.A.

Fungi of China, by S. C. Teng.

Mycotaxon, Ltd. 1996. Hardbound, xiv + 586 pp., 426 illustrations, map, portrait, index, 8–1/2x11 inches. $79.00.
Airmail price varies by country: enquire. ISBN 0-930845-05-6.
Edited by Richard P. Korf, with a foreword by Teng's daughter, Rosaline Z. Deng.
Proceeds from sales go to the Teng family.

Special for *Mycotaxon* subscribers: deduct $20 from regular price!

What reviewers say about Teng's Fungi of China:

"... one of the most valuable books on the fungi of China ever published in English." —*Mycologia* 90: 1091-1092, 1998.

"... a great big blue uncompromising brick of a book ... an instant classic. Anyone interested in identification of fungi, particularly in Asia, should get this book immediately. Mycology owes another debt to Dick Korf for his sympathetic and intelligent editing of this superb work." —*Bibliography of Systematic Mycology* 10(6): xlviii-xlix. 1998.

"The book is important for any researcher interested in the systematic relationships, diversity, and distribution of fungi on a global scale." - *New Zealand Journal of Botany* 35: 265-266. 1977.

Fungi of Northwestern China, edited by Wen-Ying Zhuang.

Mycotaxon, Ltd. 2005. Hardbound, 430 pp., 26 x 18.5 cm. US$40.00 plus postage. ISBN 0-930845-14-5.
(Please *order direct* from Wen-Ying Zhuang, PO Box 2714, Beijing 100080, China; zhuangwy@sun.im.ac.cn).

Some 3887 named species belonging to 759 genera of fungi (slime molds, plasmodiophoromycetes, chytrid, oomycetes, zygomycetes, lichens, non-lichenized ascomycetes, anamorphic fungi, rusts, smuts, and the basidiomycetous macrofungi) hitherto known from Gansu, Ningxia, Qinghai, Shaanxi and Xinjiang, China are listed. Useful references, detailed tropical distribution, and hosts or substrates are provided for each species. *Contributing authors:* Shuang-Lin Chen, Lin Guo, Shou-Yu Guo, Ying-Lan Guo, Shu-Xiao Sun, Shu-Xia Wei, Hua-An Wen, Xiao-Qing Zhang,Jian-Yun Zhuang, and Wen-Ying Zhuang.

Higher Fungi of Tropical China, edited by Wen-Ying Zhuang.

Mycotaxon, Ltd. 2001. Cloth bound, 485 pp., 26 x 18.5 cm. US$50.00 plus postage. ISBN 0-930845-13-7.
(Please *order direct* from Wen-Ying Zhuang, PO Box 2714, Beijing 100080, China; zhuangwy@sun.im.ac.cn).

Some 5056 named species belonging to 1192 genera of higher fungi hitherto known from tropical China are listed. Useful references, detailed tropical distribution, and hosts or substrates are provided for each species. *Contributing authors:* Lin Gou, Shou-Yu Guo, Ying-Lan Guo, Xiao-Lan Mao, Shu-Xiao Sun, Shu-Xia Wei, Hua-An Wen, Zhi-He Yu, Xiao-Qing Zhang, Jian-Yun Zhuang, and Wen-Ying Zhuang.

A Monograph of the Fungus Genus *Cercospora,* by Charles Chupp

Published by the author, Ithaca. 1953. Hardbound, 667 pp. 222 illustrations, octavo, $25.00. Airmail, $45.00
Proceeds from sales go to the Chupp family.
Still a classic after a half century.

Mycotaxon Cumulative Index for Volumes XLI-LX (1991-1996)

Karen D. Gettelman, Richard P. Korf, Susan C. Gruff & Ian F. Korf
Mycotaxon, Ltd. 1998. Softbound, 296 pp., 6x9 inches. $35.00. Airmail, $55.00. ISBN 0-930845-07-2.

Mycotaxon Cumulative Index for Volumes XXI-XL (1984-1991)

Richard P. Korf & Susan C. Gruff
Mycotaxon, Ltd. 1991. Softbound, 352 pp., 6x9 inches. $30.00. Airmail, $50.00. ISBN 0-930845-01-3.

Mycotaxon Cumulative Index for Volumes I-XX (1974-1984)

Richard P. Korf & Susan C. Gruff
Mycotaxon, Ltd. 1984. Softbound, 232 pp., 6x9 inches. $17.50. Airmail, $37.50.
ISBN 0-930845-00-5.

FUNGAL DIVERSITY RESEARCH SERIES (published in Hong Kong): Mycotaxon, Ltd. now ships rapidly from the U.S. to North American customers. All prices are *postpaid.* Titles include: **Marine Mycology—A Practical Approach,** Kevin D Hyde & Stephen B. Pointing (eds.), $100, 2000. **Genera of Ascomycetes from Palms,** by Kevin D. Hyde, Joanne E. Taylor & Jane Fröhlich, $100, 2000. **Palm Microfungi,** by Jane Frölich & Kevin D. Hyde, $100, 2000. **A World Monograph of *Anthostomellaa,*** by Binsheng Lu & Kevin D. Hyde, $100, 2000. **Checklist of Hong Kong Fungi,** by B.S. Lu, K.D. Hyde, W.H. Ho, H.M. Tsui, M.K.M. Wong, Yanna & D.Q. Zhou, $20, 2000. **Bio-Exploitation of Filamentous Fungi,** Stephen B. Pointing & Kevin D. Hyde (eds.), $100, 2001. **Fungi in Marine Environments,** Kevin D. Hyde (ed.), $80, 2002. **Smut fungi from New Zealand—Fungi of New Zealand volume 2,** by Kálmán Vánky & Eric H.C. McKenzie, $50, 2002. **The Genus *Mycena* in South-Eastern Australia,** by Cheryl A. Grgurinovic, $80, 2002. **Freshwater Mycology,** by C.K.M. Tsui & K.D. Hyde, $80, 2003. **Myxomycetes of New Zealand—Fungi of New Zealand volume 3,** by Stephen L. Stephenson, $50, 2003. **Microfungi of Tropical and Temperate Palms,** by Joanne E. Taylor & Kevin D. Hyde, $80, 2003. **Revision of the genus *Amphisphaeria,*** by Y.Z. Wang, André Aptroot & Kevin D. Hyde, $60, 2004. For details see the Hong Kong University website:

www.hku.hk/ecology/mycology/FDRS/index.htm

EDITORS OF MYCOTAXON

Lorelei L. Norvell, Editor-in-Chief
Pacific Northwest Mycology Service
6720 NW Skyline Boulevard
Portland, OR 97229, U.S.A

Shaun R. Pennycook, Nomenclature Editor
PennycookS@LandcareResearch.co.nz
Manaaki Whenua Landcare Research
Auckland, New Zealand

David L. Hawksworth
Book Review Editor
MycoNova, Calle Aguila 12
Colonia La Maliciosa, Mataelpino
ES-28411 Madrid, Spain

Karen D. Gettelman
Index Editor
510 Lake Blvd., Apt. 166
Davis, CA 95616
U.S.A.

Grégoire L. Hennebert
French Language Editor
32 Rue de l'Élevage
B-1340 Ottignies - LLN
Belgium

MYCOTAXON is a quarterly, peer-reviewed journal devoted to mycological taxonomy and nomenclature. All articles are reviewed by specialists prior to submission. Publication is open to everyone. Papers may be in English or in French. Summaries in those or any additional languages desired by the authors are given for longer articles. Printing is on high quality, acid-free book paper. Authors prepare their own *text-ready* files after having received critical comments from pre-submission reviewers and nomenclatural review by the Nomenclature Editor. *Instructions to Authors* (Mycotaxon **94:** 401–411, 2005) and *Guidelines for Reviewers* are updated and can be downloaded from our website at <www.mycotaxon.com>.

SUBSCRIPTION INFORMATION

All subscriptions are on an annual basis, with four quarterly volumes each year. Personal subscriptions are available at a substantially reduced rate for those who agree not to deposit their copies in a library other than their personal one within 3 years of receipt. Address **all** orders to the MYCOTAXON ORDER DEPARTMENT, P.O. Box 264, Ithaca, NY 14851-0264 USA, or your agent. Prices and availability of back volumes are posted on-line at <www.mycotaxon.com> or can be provided on request by Email from <info@mycotaxon.com>. Mycotaxon may be obtained on a journal-exchange basis. Proposals for such exchanges from journals, institutions, or individuals with difficulty in obtaining foreign currency should be addressed to the Order Department.

The annual subscription rates for 2006 (four volumes annually) are

	USA	**Foreign (IMEX air assist)**
REGULAR (multi-user)	$330	$370
PERSONAL (individual)	$150	$190

All back volumes [except volumes 22, 24, 34(2), 35, 41, 54, and 64, currently out of print] are available at $35 each by surface mail, $55 each by air mail. Volumes 1, 38, 39, 43 and 46 were reprinted in 2001; the original printing of volume 1, part 1 is provided, with parts 2 and 3 reprinted.

CUMULATIVE INDICES

Printed versions of the *Mycotaxon Cumulative Indices* for Volumes 1-20 (ISBN 0-930845-00-5), Volumes 21-40 (ISBN 0-930845-01-03), and Volumes 41-60 (ISBN 0-930845-07-02) are available (see descriptions at <www.mycotaxon.com> and order there or through your local bookstore's listings under "Books in Print"). An online *Cumulative Index to Taxa in Volumes 61-ff.* and the *Cumulative Author Index to Volumes 61-ff.* are posted at <www.mycotaxon.com>.

AVAILABILITY IN MICROFILM, PHOTOCOPY, & ELECTRONIC VERSIONS

MYCOTAXON is also partially available in *microfilm* from National Archive Publishing Company <www.napubco.com>. *Tear sheets* or *photocopies* of individual articles may be obtained from ISI Document Solution <www.isidoc.com>. Many back volumes are available as free downloads through Cyberliber <www.cybertruffle.org.uk/cyberliber/index.htm> under 'Catalogues & Journals.'

CONTACTING MYCOTAXON'S EDITOR-IN-CHIEF BY E-MAIL OR FAX

To reach the Editor-in-Chief regarding manuscripts, E-mail to <editor@mycotaxon.com> or Fax to Lorelei L. Norvell at +503.297.3296.

CONTACTING MYCOTAXON'S ORDER DEPARTMENT BY E-MAIL OR FAX

To reach the Order Department for information or placing orders, you may E-mail to <info@mycotaxon.com> or you may Fax to Orders, Mycotaxon, Ltd., May–October at +607.273.4357, and November–April at +727.321.1460.

VISIT MYCOTAXON ON THE WORLD WIDE WEB AT <www.mycotaxon.com>